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INFLUENCE OF TEMPERATURE ON NITRIFYING BACTERIA : A STUDY ON NITRIFICATION PROCESS

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CERTIFICATE

This is to certify that the present work has been done under my supervision and the work has not been submitted elsewhere for a degree.

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1. INTRODUCTION

1.1 General

With the coming up of new nitrogenous fertilizer plants and the ones already existing in India, the country is faced with the problem of treating its waste. And definitely the problem will be more and more in future. The unique characteristics of fertilizer waste is its high content of ammonia nitrogen. As high as 939 mg/1 of ammonia-nitrogen with a flow of 9.8 mgd. at Sindri Fertilizer Unit, Table I, and 1160 mg/l. with a flow 0.565 mgd at Urea Plant Kanpur, Table II have been reported (1)(2).

The harmful effects of taking these wastes untreated to lakes or streams are well-known. Nitrogen even in small concentrations acts as a fertilizer and encourages heavy growth of algae and other planktons. Some of the ill-effects of excessive algal growth have been summarized by Lackey (3) as

(a) Growth of toxic algae. He quoted an example of Oysters being adversely affected by the growth of an undesirable species of algae.

(b) Aesthetic effect. Due to growth and decay of algae natural waters get a soapy appearance, and acquire unpleasant tastes and odors.

(c) Deoxygenation of waters. The photosynthetic plants produce supersaturation in the daytime and oxygen depletion at night. Such extremity of environment is not suitable for many aquatic lives. More serious problem is created when, after reaching a high level of growth, they start dying possibly due to their own excreted metabolites. Oxygen depletion due to such decomposition is fast and there is no replacement from photosynthesis.

TABLE 1

CHARACTERISTICS OF WASTEWATER AT SINDRI FERTILIZER UNIT, SINDRI

Description	Period	No. of Readings	Constituents	Analysis in ppm Max.	Min.	Ave.
Stream I	May to June, 1965	15	Total Phenol	8.0	0.2	1.6
			Cyanide as CN	5.0	0.05	1.5
			Amm. Nitrogen	238	7.0	88
			Suspended Solids	7190	960	3369
			Oil	24	1.0	8.2
			Flow in mgd	3.2	1.2	2.1
Stream II	May to June, 1965	15	Phenol	14.0	0.1	0.7
			Cyanide	3.2	0.4	1.2
			Amm. Nitrogen	939	242	313
			Suspended Solids	790	97	398
			Oil	29	2.0	11.5
			Flow in mgd	9.8	6.2	7.8

Still another problem in streams only has been encountered by Sawyer (4). In shallow soft-bottom streams aquatic forms get rooted and so dense as to choke the flow of water and cause flooding the upstream reaches. In some cases they themselves take up oxygen in the night time and the oxygen-depleted water travels some distance. Unless reaeration in the downstream is sufficient, many fishes may be killed.

Apart from this, it has been found that drinking waters with high nitrate content often cause methamoglobinemia in infants. In some places of United States, a maximum of 20 mg/l. NO_3-N is permitted in public water supply (5) And, since biological oxidation of ammonia leads ultimately to nitrate, high concentration of the

TABLE 2

ESTIMATED CHARACTERISTICS OF UREA PLANT, KANPUR

Item	kg/hr	ppm
Volume 107 M^3/hr		
Temperature 45°C		
Urea	114.7	1065
NH_3	149.3	1410
CO_2	200.5	1860
Other dissolved solids	136.4	1280
Total dissolved solids	251.1	2345
Suspended solids	-	-
Total Solids	251.1	2345
Ammonical Nitrogen		1160

former also is not desirable.

Lastly it is expected that ammonia at high concentration will be toxic to fish life. Even after dilution of fertiliser waste in a stream, ammonia content may be high enough to cause hazard.

Possibility of satisfactory treatment of fertilizer waste by our conventional biological processes like activated sludge seems to be remote because of high ammonia content of this waste. Various other methods for the removal of nitrogen are being considered and investigated these days. A classification has been given by Eliassen et al (6). Table III shows a list of these methods together with their removal efficiency, estimated running cost and the form of the ultimate waste to be disposed of. Out of all these methods a few need elaboration here.

TABLE 3

COMPARISON OF NITROGEN REMOVAL METHODS

Process	Classification*	Removal efficiency per cent	Estimated removal cost $/mil gal	Type of waste to be disposed	Remarks
Ammonia stripping	C*	80-98	9-25		Removal effi ncy is base ammonia nit en only.
Conventional Biological Treatment	B*	30-50	30-100	sludge	
Anaerobic Denitrification	B*	60-95	25-30	none	
Algae Harvesting	B*	50-90	20-35	liquid & sludge	Large land area requir
Ion Exchange	C*	80-92	170-300	Liquid	Efficiency & cost depend degree of p reatment, c filt., etc.
Electrochemical Treatment	C*	80-95	4-8	liquid & sludge	
Electrodialysis	C*	30-50	100-250	liquid	Costs based 1-10 mgd, ca city solid concentratio 1000 mg/l.
Reverse Osmosis	P*	65-95	250-400	liquid	
Distillation	P*	90-98	400-1000	liquid	
Land Application	P*			none	

* B = Biological, C = Chemical, P = Physical

Biological treatment - In any biological treatment process organisms need nutrients like nitrogen and phosphorous besides the main organic food. All these organic matter, nitrogen and phosphorous are synthesized into cell material which can be clarified later. The feasibility of this method will depend largely on the amount of organic substance available. If the gross composition of biological growth is assumed to be $C_5H_7NO_2$., organic matter needed to balance the nitrogen of fertilizer waste will be too high to be of any practical value.

Algae harvesting - This is accomplished using specially designed shallow ponds. Soluble and colloidal nitrogen are bound to algal cell tissue. Cell growth may be represented by the followi equation

$$aCO_2 + cNO_3^- + ePO_4^{\equiv} + (c+3e)H^+ + \tfrac{1}{2}(b-c-3e)H_2O \rightleftharpoons$$

$$C_a H_b N_c O_d P_e + (a + \frac{b}{4} + \frac{5c}{4} - \frac{d}{2} + \frac{5e}{4})O_2$$

Disadvantages with this process are large land requirement and disposal of algae.

Ammonia stripping - In wastewater ammonium ions exist in equilibrium with ammonia and hydrogen ions as shown by the equation

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

As the pH is raised about seven, the equilibrium is shifted to the right, and by agitating the water with the help of air, ammonia can be liberated. Needless to say this method will be economically feasible only when ammonia concentration in the waste in quite high. As for the fertilizer waste, ammonia stripping can be used as a primary treatment process.

Electrochemical treatment - In this method sewage is mixed with sea-water and passed into a single cell with carbon electrodes. Because of the higher density of sea water it accumulates at the bottom around anode and the mixture at the top around cathode. Current raises the pH at cathode, thus precipitating phosphorous and ammonia as $Ca_3(PO_4)_2$ and $M_gNH_4PO_4$ along with $M_g(OH)_2$. Hydrogen bubbles, generated at cathode, lift the sludge to the surface where it can be skimmed and chlorine at the anode provides for disinfection.

Land application - Removal of nitrogen by this method is mainly due to physical adsorption of ammonium ions by soil particles. Nitrate however passes unchanged through the soil system.

Nitrification - denitrification process - By this time this system has drawn considerable attention. Nitrification unit utilizes some aerobic autotrops which derive their energy from the oxidation of ammonia and nitrite. The denitrification unit employs some anaerobes which are capable of reducing nitrate and nitrite to nitrogen and nitrous oxide which ultimately escape into atmosphere.

1.2 Scope of study

The present work relates to one aspect of nitrification and that is "influence of temperature on Nitrification process". It is generally believed that in any biological system velocity of reaction can be improved with increasing temperature. Bacteria can withstand a limited range of temperature with an intermediate temperature of maximum growth. And also is noted the fact that fertilizer

industry often throws hot wastes. Some waste streams from Urea Plant, Kanpur is as hot as to reach 45°C, ~~Table 2~~. It is therefore possible to segregate or mix the various streams in a manner to achieve a temperature where bacteria can work faster. This eventually will reduce detention period requirement, and a smaller nitrification unit may be adequate. It is therefore proposed to study the effect of temperature on the metabolic activity of nitrifying bacteria.

2. LITERATURE REVIEW

2.1 Fertilizer Waste and its Treatment

As a method of treatment for nitrogenous fertilizer industry waste, Nitrification denitrification process is drawing attention in recent times. It has high potentiality to convert all the combined nitrogen to elemental nitrogen which escapes into atmosphere.

"Nitrification" is the process where ammonia from the breakdown of proteins or from other sources is oxidized biologically to nitrate. The process goes in two steps. First ammonia is oxidized to nitrite ($NH_3 + 3/2\ O_2 \rightarrow HNO_2 + H_2O$) by a group of bacteria, Nitrosomonas europaea being the most common one. Later nitrite is oxidized to nitrate ($HNO_2 + \frac{1}{2}O_2 \rightarrow HNO_3$) by other group of bacteria, Nitrobacter winogradskii being the most common one. Table 4 and 5 summarize informations regarding Nitrosomonas and Nitrobacter (7).

TABLE 4

NITROSOMONAS EUROPAEA

Order : Pseudomonadales　　　Family : Nitrobacteraceae

Rods 0.9 to 1.0 by 1.1 to 1.8 μ, occuring singly, rarely in chains of three to four. Possess a single polar flagellum 3 to 4 times the length of the rods, or rarely one at either end.

Grow readily in aquous medium without organic matter and containing ammonium sulphate, potassium phosphate and magnesium carbonate. The cells accumalate in soft masses around the particles of magnesium carbonates at the bottom of the flask. The liquid is occasionally turbid through development of motile swarm cells or monads.

Small, compact, sharply defined colonies brownish in colour on silica gel.

Aerobic.
Strictly autotrophic.
Source : Soils.
Habitat: Presumably widely distributed in soil.

TABLE 5

NITROBACTER WINOGRADSKII

Order : Pseudomonadales   Family : Nitrobacteraceae

Short, Non - motile rods with gelatinous membrane, 0.6 to 0.8 by 1.0 to 1.2 microns. Do not stain readily. Gram-negative.

Can be cultivated on media free of organic matter. Sensitive to certain organic compounds.

Washed agar colonies : In 7 to 10 days very small light, brown, circular to irregular colonies, becoming darker.

Silica gel : Colonies smaller but more dense than those on washed agar.

Washed agar slant : In 7 to 10 days, scant, grayish streak.

Inorganic solution medium : After 10 days flocculent sediment. Sensitive to ammonium salts under alkaline conditions.

Nitrite is oxidized to nitrate.

Aerobic.
Strictly autotrophic.
Optimum temperature : between 25°C and 28°C
Source : Soil
Habitat: Presumabily widely distributed in soil.

"Denitrification" the process of forming nitrite N_2O or N_2 from nitrates was observed as early as in 1868 by Schloesing. "In its essence the reduction of nitrate is the use of oxygen of nitrate as a hydrogen acceptor. Consequently a source of combined hydrogen, i.e., organic matter, and a limitation on the supply of free oxygen, are needed".(8)

The following are the intermediate steps in the Denitrification process.

$$\underset{\text{nitrate}}{HNO_3} + 2(H) \rightarrow \underset{\text{nitrite}}{HNO_2} + H_2O \qquad \ldots \text{(i)}$$

$$HNO_2 + 2(H) \rightarrow \tfrac{1}{2}(H_2N_2O_2) + H_2O \quad \ldots \quad (ii)$$

nitrite hyponitrite

$$(H_2N_2O_2) + 2(H) \rightarrow N_2 + 2H_2O \quad \ldots \quad (iii)$$

nitrogen (gas)

Hyponitrite is unstable and can undergo breakdown differently.

$$(H_2N_2O_2) \longrightarrow H_2O + N_2O \quad \ldots \quad (iv)$$

nitrous oxide (g)

There is evidence of nitrous oxide also being reduced to nitrogen gas.

$$N_2O + 2(H) \longrightarrow N_2 + H_2O \quad \ldots \quad (v)$$

Ps. denitrificans which ordinarily reduces nitrate to nitrogen, is able to synthesise "adaptive enzyme" for the reduction of N_2O (9). *Ps.stutzer* and *Ps. pyocyanaea* can rapidly decompose N_2O while Denitrobacillus seems to act slow (8).

Reduction of nitrate to ammonia is also possible. The reducing bacteria include a wide variety of groups like Azotobacter, Radiobacter, *Cl. welchii*, Desulfovibrio, *Denitrobacillus licheniformis* and *Bac. subtilis*. In the case of *Cl. welchii* (10) even hydrogen gas can act as a hydrogen donor. The reaction goes like

$$HNO_3 + 4H_2 \longrightarrow NH_3 + 3H_2O \quad \ldots \quad (vi)$$

2.2 Effect of Temperature on Bacterial Growth

Rate of any chemical reaction is enhanced by increase in temperature. And it is not difficult to imagine that bacterial growth also is affected by temperature in a similar way when one considers that enzymatic reactions are purely of chemical nature. However, growth is an outcome of a sequence of reactions and one must expect a certain complexity in the variation of growth-rate with temperature. Nevertheless, it has been observed by many workers (11)(12)(13) that agglomeration of unorganised systems leads to an approximate accordance with the Arrhenius Law

$$\frac{d \ln K}{dT} = \frac{E}{RT^2}$$

where K is the velocity of reaction, E the energy of activation, R the Universal gas constant and T temperature in absolute degree.

Barber's work (11) is most widely quoted for effect of temperature on bacterial growth. He plotted mean generation time as a function of temperature. In lower temperature ranges the rate of growth of bacteria increases rapidly with increase in temperature, the change being about two to three fold for each 10°C rise in temperature, Fig. (1). Upto 35°C generation time drops, between 35° and 45° it remains almost constant, and between 45° and 50° it increases very slowly. At still higher temperatures growth fails complitely and the cells are killed.

Ingraham (14) has studied a psychrophile and a mesophile, and has shown the essential validity of Arrhenius equation, Fig.(2). He plotted logarithm of growth-rate against inverse of temperature in absolute degree. Both the curves were linear over the lower temperature range, giving a temperature characteristics of 14,200

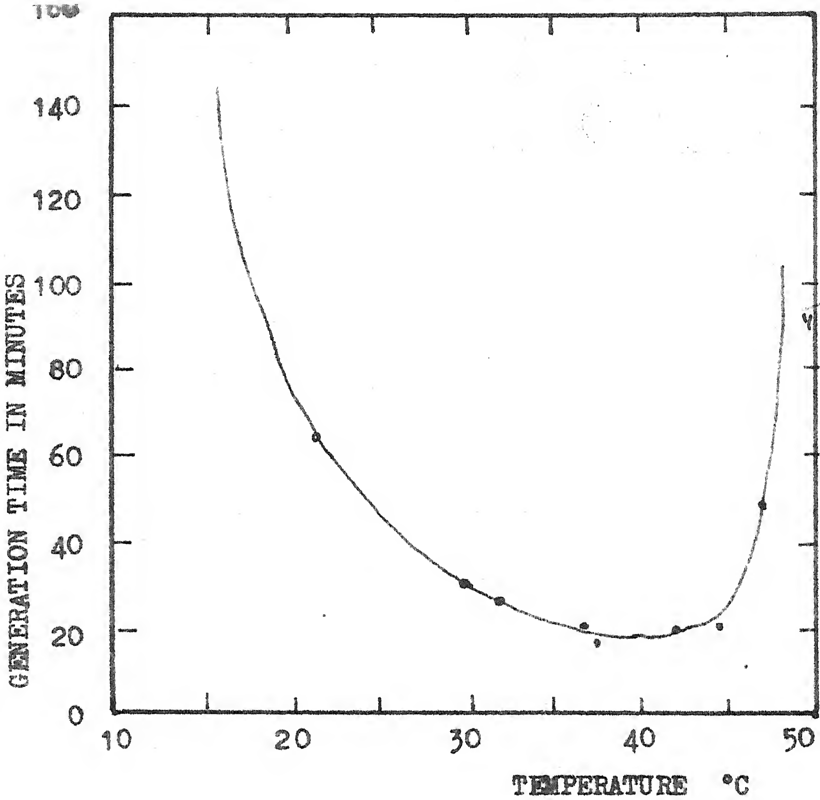

FIG. 1. GENERATION TIMES OF B.COLI AFTER BARBER (11)

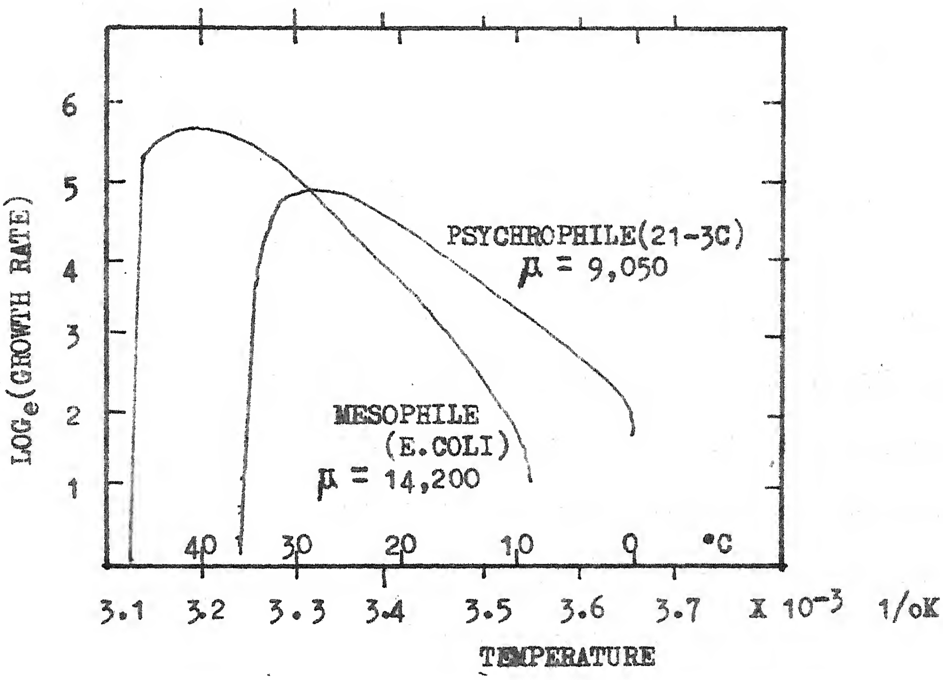

FIG. 2. ARRHENIUS PLOT OF GROWTH RATES AFTER INGRAHAM (14)

cal/mole for the mesophile (E.coli), and 9,050 cal/mole for the psychrophile. At higher temperatures growth rate was falling rapidly.

It is evident from the works of Barber, Ingraham and others that bacterial multiplication-rate does not increase when a certain temperature is reached, and after that the rate falls down rapidly. This signifies the difference between bioligical and chemical reactions. While any chemical reaction can be made faster infinitely by increasing temperature, biological one cannot be made so. This limitation in the case of biological growth is stated to be due to inactivation of enzymes, and coagulation and denaturation of cell material at high temperatures. Thus high temperature brings in a second series of reactions, viz., coagulation of protein etc.

A complete and logical formulation for growth constant has been proposed by Hinshelwood (15). The equation is

$$\mu_m = \mu_1 e^{-E_1/RT} - \mu_2^{-E_2/RT}$$

where μ_1 μ_2 are constants, E_1 E_2 are known as temperature characteristic or Arrhenius constant, T is temperature °K and R is the universal gas constant.

The first term represents rate of growth and the second term rate of death. In the lower temperature ranges (around 10° to 35°C) the first term is much more predominant so that growth rate increases with temperature. With further increase in temperature the second term becomes appreciably large with the result that growth rate does not change much, and later falls down rapidly.

2.2.1 Effect of Low Temperature and the Psychrophiles

The minimum temperatures at which bacteria can multiply is difficult to determine accurately, because there is no sharp declination in the growth rate at low temperatures. Moreover, with decrease in temperature, generation time goes on extending very much. In fact, Ingraham (14) has observed generation time as long as 103 hrs. For this reason some suggest that in determining the minimum temperature incubation be continued for a week or so.

Many bacteria are killed by cooling down to 0°C. The mechanism by which freezing kills bacteria is believed to be the disruption of cell organization by the formation of relatively large ice crystals. Yet there are many who can survive at such temperatures (8). B.Coli was found to survive better at -20°C than at -2°C. In cream stored for a year at -20°C the total count was reduced from 2½ million per c.c. to only 680,000 per c.c. Milk-souring organism, Sc.lactis, in gelatin or in water, survived 111 days' immersion in liquid air (-191°C) or 45 minutes in liquid hydrogen (-253°C), and subsequently grew at the same rate as controls. Salmonella typhosa have been isolated from ice cream kept for two years and four months at -20°C.

Psychrophiles, the so called "cold loving" bacteria, which predominate at low temperatures over others, can even grow at 0°C. How these bacteria can grow at this temperature or rather the mechanism of their survival has been of considerable interest in recent years. Ingraham (16) has discussed and evaluated the merits of some of the explanations.

(i) Fig. (2) shows the basic difference in the pattern of curves for Psychrophiles and Mesophiles. The slope of the linear portion of the curve is lesser for Psychrophiles so that its projection reaches 0°C on the positive side of growth rate. Now that the slope represents activation energy, it has been well assumed by some people that the psychrophily property lies in the basic nature of their enzymes of having lesser activation energy. The theory, however, has been disapproved by Ingraham and Bailey (17) who studied cell-free enzymes from Psychrophiles and Mesophiles and found them identical with respect to temperature characteristics.

(ii) The explanation that Psychrophiles contain larger amounts of enzyme does not seem to hold ground. "Because at about 8°C the growth rate of Psychrophiles is more than 100 times faster than the growth rate of Mesophiles, and it hardly seems possible that they could contain more than 100 times as much enzyme".

(iii) Brown and also Ingraham and Baily have observed that Psychrophiles have less temperature characteristic than Mesophiles for the oxidation of certain substrates. But the difference disappears when the broken cells are tested. They attribute the psychrophily character to the "intact cells" rather than the constituing enzymes.

In frozen foods, ice crystals out separately, leaving a relatively concentrated solution. Thimann (8) considers it possible that bacteria can survive in this aquous phase which does not solidify at ordinary freezing temperatures.

2.2.2 Moderate Temperature and the Mesophiles

The most widely and commonly encountered bacteria are the Mesophiles. They cover a temperature range of more or less 10°C to 45°C with optima around 37°C. Nitrosomonas and Nitrobacter, the bacteria that have been studied here are Mesophiles. Studies of Knowles et al (18) reveal that growth of nitrifying bacteria is considerably affected by temperature. Over a temperature range of 8°C to 23°C which they studied, growth constant of Nitrosomonas increased about 95 percent per degree centigrade rise in temperature, and that of Nitrobacter increased about 5.9 per cent per degree. The equations of best fit as found by them are

$$\log_{10} \mu = 0.0413\ T - 0.944 \qquad (T \text{ in } {}^{\circ}C)$$

in the case of Nitrosomonas and

$$\log_{10} \mu = 0.0255\ T - 0.492$$

in the case of Nitrobacter.

Mesophilic bacteria are killed by exposure to temperature of 50° to 60°C. Proteins the constituing material of cell cannot withstand this temperature, and are denatured and coagulated.

2.2.3 Effect of High Temperature and the Thermophiles

Thermophilic bacteria are found in nature in hot springs, sewage, the intestinal contents of various animals, in the air, in milk and in the soil. Some strains cause spoilage of canned foods which are sterilized at elevated temperatures. Thermophiles are particularly troublesome in the dairy industry since they may grow most rapidly at pasteurization temperatures (62°C or 72°C). As a rule the thermophiles encountered are aerobic sporeformers such as Bacillus coagulans or B.stearothermophilus but Lactobacillus thermophilus may also be a source of trouble (19).

Much effort has been expended to bring out the mechanism by which Thermophiles can grow and survive at temperatures where proteins are generally degenerated.

Allen (20) has put forward her theory of "dynamic nature of thermophily". All the proteins which are denatured at high temperature are substituted and synthesised back rapidly "much as it would be possible to keep a leaking ship afloat by rapidly pumping water from her hold" (16).

A second theory that Thermophiles contain proteins with greater heat stability has drawn much support. In fact, Militzer and his co-workers (21) in a series of experiments, have confirmed the theory. They have examined the corresponding enzymes from a Mesophile and <u>Bacillus stearothermophilus</u> and found that while malic dehydrogenase from the Thermophile is stable at 65°C, the corresponding one from the Mesophile gets inactivated within 10 minutes at the same temperature. Also an apyrase and a pyrophosphatase from the Thermophile are found to have marked heat stability. On the other hand, it has also been shown that pyruvic acid oxidation system of the same Thermophile gets inactivated, but not when in the intact cell, at 65°C. So it can be infered that most enzymes, but not all, from Thermophiles have greater heat stability than those of Mesophiles.

Koffler et al (22) have studied non-enzymic proteins also like "flagellins" the fibrous proteins that constitute flagella. They disintegrate and lose their structure on heating, the change being measurable by viscosity determinations. Flagellins from Thermophiles seem to be stable up to 70°C whereas those of Mesophiles are denatured at temperatures over 50°C.

Resistance of spores to heat has been attributed to their

low moisture content. Dry proteins are usually quite resistant to high temperatures. Ovalbumin has been stated to retain its solubility in cold water even after heating at 170°C in the dry state. Partially dry preparations or highly concentrated solutions tolerate intermediate temperatures (8).

Heat-resistance is often enhanced by the protective action of proteins, fats, and other colloids in the suspending medium. "Temperature required to kill lactic acid bacteria is much greater in cream or milk than in peptone water". Proteinase from <u>Ps.fluorescens</u> is well protected by broth medium at 60°C, while at 80°C it is denatured by 80 percent if in water, and only 27 percent in the presence of casein.

2.3 <u>Growth Kinetics</u>

Under favourable conditions of moisture, nutrition, pH and temperature bacterial cells grow in size and divide into daughter ones. Growth rate however varies with increasing time. The number of bacteria increases at first logarithmically, and then the growth rate goes on diminishing until a stationary phase is reached. The bacterial number is limited to this stationary phase possibly due to depletion of nutrients or accumulation of metabolites or non-availability of space. Later the bacteria die away.

Growth rate has been expressed mathematically by Quastel (23) for nitrifying bacteria as

$$\frac{dX}{dt} = \mu_m X \qquad \ldots \qquad \ldots \qquad (1)$$

where X is the number of bacteria at any time t,

and μ_m is growth constant.

Knowles et al (18) included in this equation Monod's often used substrate term $\mu_m \frac{S}{K_s+S}$ where S is the substrate concentration and K_s is the Michaelis constant, to take care of retarding effect on growth due to reduction in substrate concentration. However, usually Michaelis constant is pretty low. In their study, it rarely exceeded 8.0 mg/l. amm-N for Nitrosomonas, and 8.0 mg/l. nitrite-N for Nitrobacter. Consequently, by keeping substrate concentration at high level, a simplification like equation 1 can be applied.

Equation 1 on integration gives,

$$\log_e \frac{X}{X_o} = \mu_m . t \qquad \ldots \qquad \ldots \qquad (2)$$

where X_o is the number of bacteria initially present.
If "p" is the rate of mean metabolic activity of each cell so that "pX" is the rate of metabolism which may be represented by rate of oxygen uptake, for X cells.

Equation 2 can be re-written as

$$\log_e \frac{pX}{pX_o} = \mu_m . t \qquad \ldots \qquad \ldots \qquad (3)$$

$$\text{or} \quad pX = pX_o . e^{\mu_m t} \qquad \ldots \qquad \ldots \qquad (4)$$

Now pX or rate of oxygen uptake can be written as $\frac{dy}{dt}$, so that

$$\frac{dy}{dt} = pX_o . e^{\mu_m t} \qquad \ldots \qquad \ldots \qquad (5)$$

which on integration gives

$$y = \mu' (e^{\mu_m t} - 1) \qquad \ldots \qquad \ldots \qquad (6)$$

where $\mu' = \frac{pX_o}{\mu_m}$ = a constant.

For solving for μ_m in the above equation, Quastel et al simplified this equation further by assuming $e^{\mu_m t}$ is much larger compared to unity. This simplification is not well justified when t is small, and one considers that μ_m may be as low as 0.2 per day at 8.3°C for Nitrosomonas and 0.5 per day at 8.3°C for Nitrobacter(18)

As a method of solving for μ_m, it is proposed here to use finite difference method in a similar way as has been used by Fuzi Moto (24) for solving for BOD rate constant. If at every 'h' unit interval of time, oxygen uptake readings are noted, then,

$$y_t = \mu'(e^{\mu_m t}-1) \quad \ldots \quad \ldots \quad (7)$$

and $$y_{t+h} = \mu'(e^{\mu_m(t+h)}-1) \quad \ldots \quad \ldots \quad (8)$$

Substituting for $e^{\mu_m t}$ in equation 8 from equation 7,

$$y_{t+h} = e^{\mu_m h}.y_t + \mu'(e^{\mu_m h}-1) \quad \ldots \quad \ldots \quad (9)$$

The variables y_{t+h} and y_t result in a linear plot and the slope is a measure of μ_m.

3. MATERIALS AND METHODS

3.1 Preparation of Inoculum:

Fresh sewage settled for half an hour was used as a source of nitrifying bacteria. A one liter beaker was taken and in it was provided a sand bed of depth about 7 cms and of grain size 18/25 mesh. The sewage as poured over the bed to a volume of about 700 ml. and was continuously aerated with the help of a sparger. The reactor was daily fed with a 30 ml. solution of 0.02 M ammonium chloride. The feed solution was buffered to pH 8.1 with phosphate buffer of one tenth molarity containing nutrients as described later. Initially nitrification proceeded at a slow rate and the nitrifying bacteria built up profusely within a week as indicated by the extent of nitrification.

3.2 Substrate for Growth:

Ammonium chloride of 0.05 molarity and sodium nitrite of 0.05 molarity were chosen as substrates for Nitrosomonas and Nitrobacter respectively. The choice of concentration was somewhat arbitrary. 0.1 M NH_3-N is inhibitory to Nitrosomonas and 0.069 M NO_2-N is inhibitory to Nitrobacters (25). Furthermore, at very low concentrations of substrate, growth constant becomes concentration-dependent, which is not desired. Therefore intermediate values of substrate concentration were chosen.

The main substrate was supplemented with nutrients table 6 used by Siddiqi et al. (26) and buffered with 0.1 molar phosphate buffer. The pH was maintained at 8.1 in accordance with the observations of Mojumdar (25) who found optimum pH range for Nitrosomonas as 7.8 to 8.3 and for Nitrobacter as 8.0 to 8.5.

TABLE 6

INORGANIC MEDIA

Inorganic Salts	Amount
$Fe\ Cl_3 . 6\ H_2O$	0.125 mg/l.
$Mg\ SO_4 . 7\ H_2O$	25.00 mg/l.
K_2HPO_4	3.00 mg/l.
$Ca\ CO_3$	50.00 mg/l.
$Na\ HCO_3$	250.00 mg/l.

3.3 Measurement of Growth:

Growth - rates of Nitrosomonas and Nitrobacter were followed in Warburg Respirometer. Separate flasks were used for ammonia and nitrite substrates. Respiration in two replicates for each case together with a thermobarometer were observed. A few grams of sand granules were taken out from the nitrifying reactor, washed several times with distilled water to wash away ammonia, nitrite and nitrate, and dried by pressing between filter papers. These "active granules" were then mixed uniformely with blank sand particles. Each reactor flask was filled with four grams of this mixed soil and two millikiters of feed solution. This combination provided for adequate oxygen supply by diffusion through the liquid. Shaking was adjusted to about 48 oscillations per minute with an amplitude of 4 cms. The Warburg flask constants were calculated by using the formula (27).

$$x = h . \frac{V_g . \frac{273}{T} + V_f . \alpha}{P_o} \quad \ldots \quad \ldots \quad (10)$$

$= h.K$

where,

X = oxygen uptake in μl.,

h = change of manometric reading in mm.,

V_g = volume of gas in the flask in μl.,

V_f = volume of fluid in the flask in μl.,

T = temperature in ^{o}K,

α = solubility of oxygen in ml of O_2 dissolved per ml. water,

P_o = standard pressure = 10,000 mm of Brodie's fluid,

K = flask constant.

In measureing V_g, volume of sand particles taken in the flask, besides the volume of liquid, was excluded from volume of flask. (see appendix A for flask constants table 7 and solubility of oxygen in water table 8 at different temperatures).

The respiration rates were determined at ten different temperatures in the range between 20°C and 41.5°C.

3.4 <u>Analytical Techniques:</u>

At the end of a run final concentrations of ammonia, nitrite and nitrate were determined chemically.

<u>Ammonia:</u> Ammonia was determined spectrophotometrically (28) by using Nesslers Reagent. The standard calibration curve is shown in Fig. 3.

<u>Nitrite:</u> Sulfanilic acid-napthylamine hydrochloride method (28) was followed for nitrite determination. The standard curve is shown in Fig. 4.

<u>Nitrate:</u> Nitrate was determined photometrically using Brucine sulfanilic acid (28). The standard curve is shown in Fig. 5.

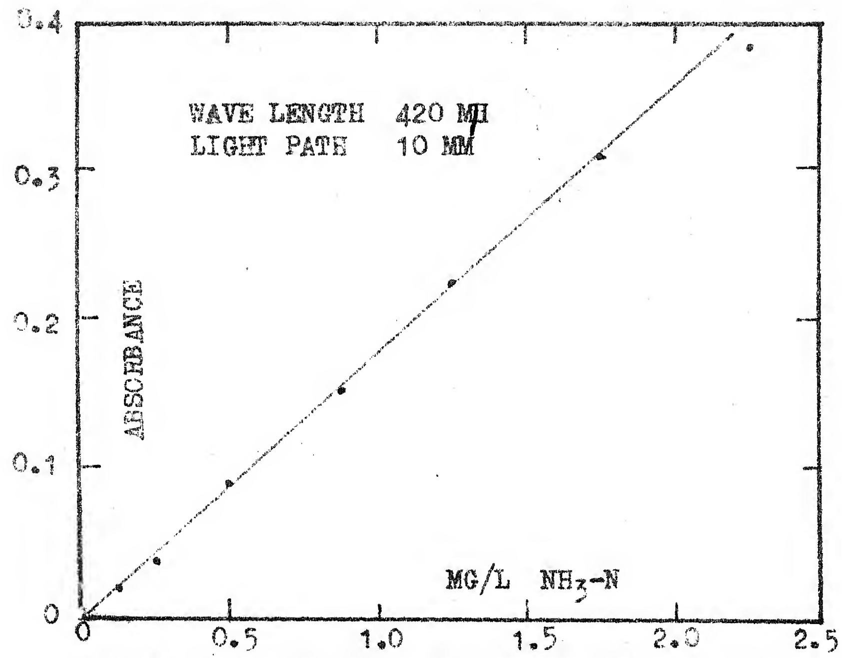

FIG.3. STANDARD CURVE FOR ESTIMATING AMMONIA

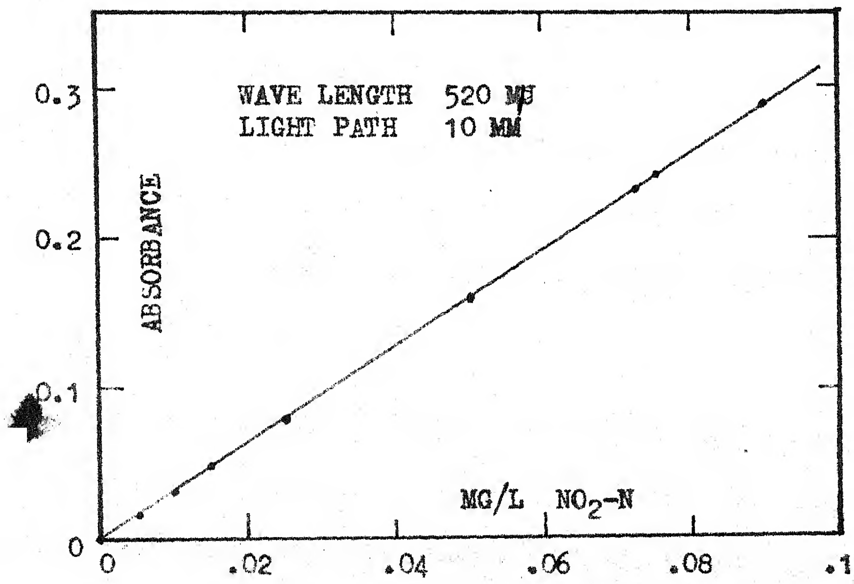

FIG.4. STANDARD CURVE FOR ESTIMATING NITRITE

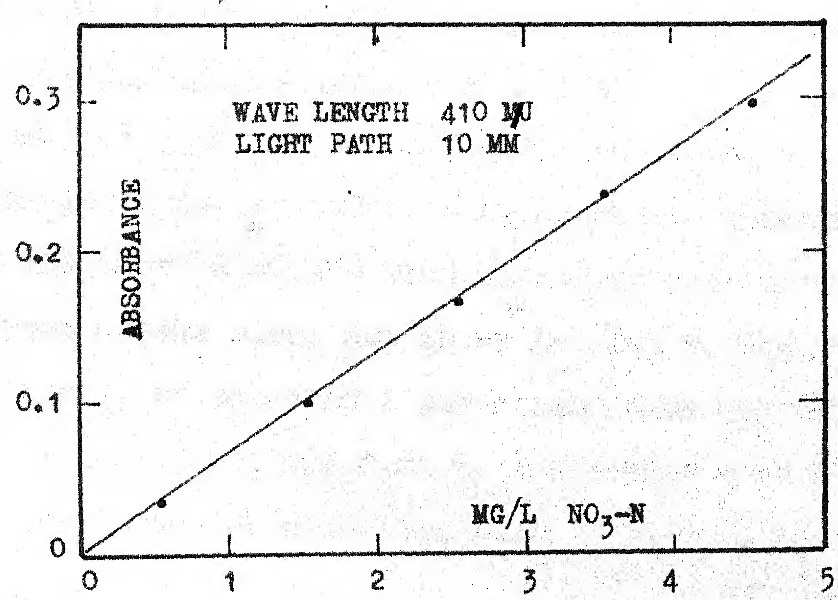

FIG. 5. STANDARD CURVE FOR ESTIMATING NITRATE

4. RESULTS & DISCUSSIONS

4.1 Preliminary Observations:

4.1.1 Approach the Problem

Kinetics of bacterial growth has been studied by many workers in different ways. Ingraham (14) utilized "turbidity of the medium" as a measure of growth. In studying E.Coli in trypticase-soy medium he observed, at intervals of time, turbidity photometrically at 660 mμ. The period in which turbidity doubled is obviously the generation time.

Knowles et al. (18) utilized "exhaustion of substrate" and "production of metabolites" as a measure of growth. They studied nitrifying bacteria, and followed the concentrations of ammonia, nitrite and nitrate with time. They obtained growth constants by fitting the data in an equation whose differential form is $\frac{dx}{dt} = \mu_m . \frac{S}{K_s + S} . x$

In the present study "oxygen uptake" or the "respiration" of bacteria has been taken as a measure of growth. Oxygen uptake of nitrifying bacteria has been observed at intervals of time in the Warburg Respirometer. This method is supposed to be more accurate than any of the two methods previously described.

4.1.2 Effect of Substrate Concentration

Since some workers reported that growth characteristics of nitrifying bacteria are affected by substrate concentration a trial run was made at 28.1°C with different concentrations of ammonia. Oxygen uptake plots are shown in Fig. 6. Magnitude of growth constant u_m of equation 1 was around 0.63 day^{-1} for concentrations of 0.05, 0.03 and 0.01 M, indicating that growth constant is unaffected at these levels of substrate concentration.

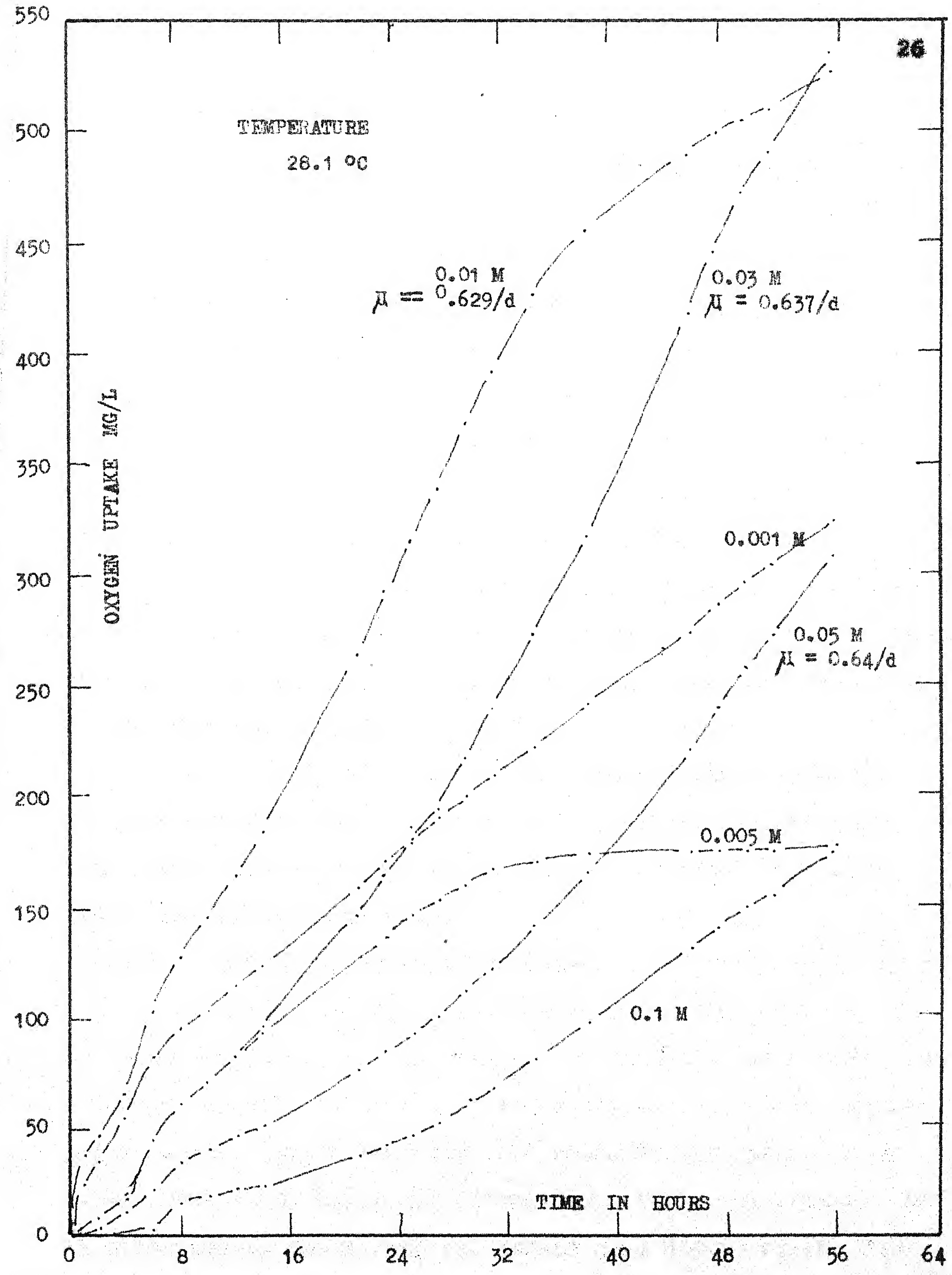

FIG. 6. OXYGEN UPTAKE FOR OXIDATION OF AMMONIA AT DIFFERENT CONCENTRATIONS

At 0.1 M ammonia, oxidation was much less; possibly this concentration of ammonia is inhibitory to nitrifying bacteria.

At concentrations .001 M and .005 M ammonia, growth constant is pretty low as compared to those at higher concentrations. This shows that around and below 0.005 M (70 mg/1.) NH_3-N, growth of Nitrosomonas is retarded with the depletion of substrate. This has been further confirmed by the nature of curve for 0.01 M (140 mg/1.) NH_3-N in the same fig. The slope of the curve started dropping distinctly when oxygen consumption was around 400 mg/1. or when NH_3-N concentration reduced to 25 mg/1. Here it may be recollected that Michaleis constant as found by Knowles for Nitrosomonas is around 8.0 mg/1. of NH_3-N . And in that case it is not surprising why the curve started falling at around 25 mg/1.

However, in all the studies reported here, starting ammonia concentration is 0.05 M (700 mg/1.) so that even if the concentration reduces to as low as 100 mg/1. there will not be any appreciable error.

4.1.3 <u>Effect of Active Granules</u>:

At 40°C Fig. 15 in the flasks containing nitrite, all the sand particles present were active granules taken from 'seed reactor'. It will be seen that after about 10-12 hours, oxygen uptake varied almost linearly with time. This indicates that after about 10-12 hours the Nitrobacters could not multiply or in other words, death rate and growth rate became equal. This situation might have been created by the over-crowding of bacteria or rather non-availibility of space for their growth.

Therefore in all the studies, active granules constituted only about one-tenth of the total sand particles.

4.2 Effect of Temperature:

Table 9 to table 18 (Appendix B) show oxygen uptake by Nitrosomonas and Nitrobacter at different temperatures as recorded in Warburg Respirometer. At the end of each table final concentrations of ammonia, nitrite and nitrate are mentioned. Corresponding plots of oxygen uptake against time are shown in Fig. 7 through Fig. 16.

To evaluate growth constants, finite difference method as described on page 20 has been employed throughout. All such plots have not been shown, instead a sample plot Fig. 17 is shown in Appendix C. Table 19 summarizes these results.

TABLE 19

GROWTH CONSTANCES

Temp. °C	Nitrosomonas Reactor 1	Nitrosomonas Reactor 2	Nitrosomonas day^{-1} average	Nitrobacter Reactor 1	Nitrobacter Reactor 2	Nitrobacter day^{-1} average
20°	.279	.232	.255	.1312	.135	.133
22°	.281	.324	.302	.212	.1905	.201
25°	.48	.484	.482	.24	.25	.245
28°	.62	.654	.637	.322	.333	.327
32°	.9	.905	.903	.35	.494	.4
34°	.922	.68	.777	.635	.795	.71
36°	.688	.39	.539	.85	.745	.8
38°	.482	.3545	.418	1.09	1.19	1.14
40°	.119	.12	.1195	0*	0*	0*
41.5°	-	-	-	.41	.431	.42

* with active granules only.

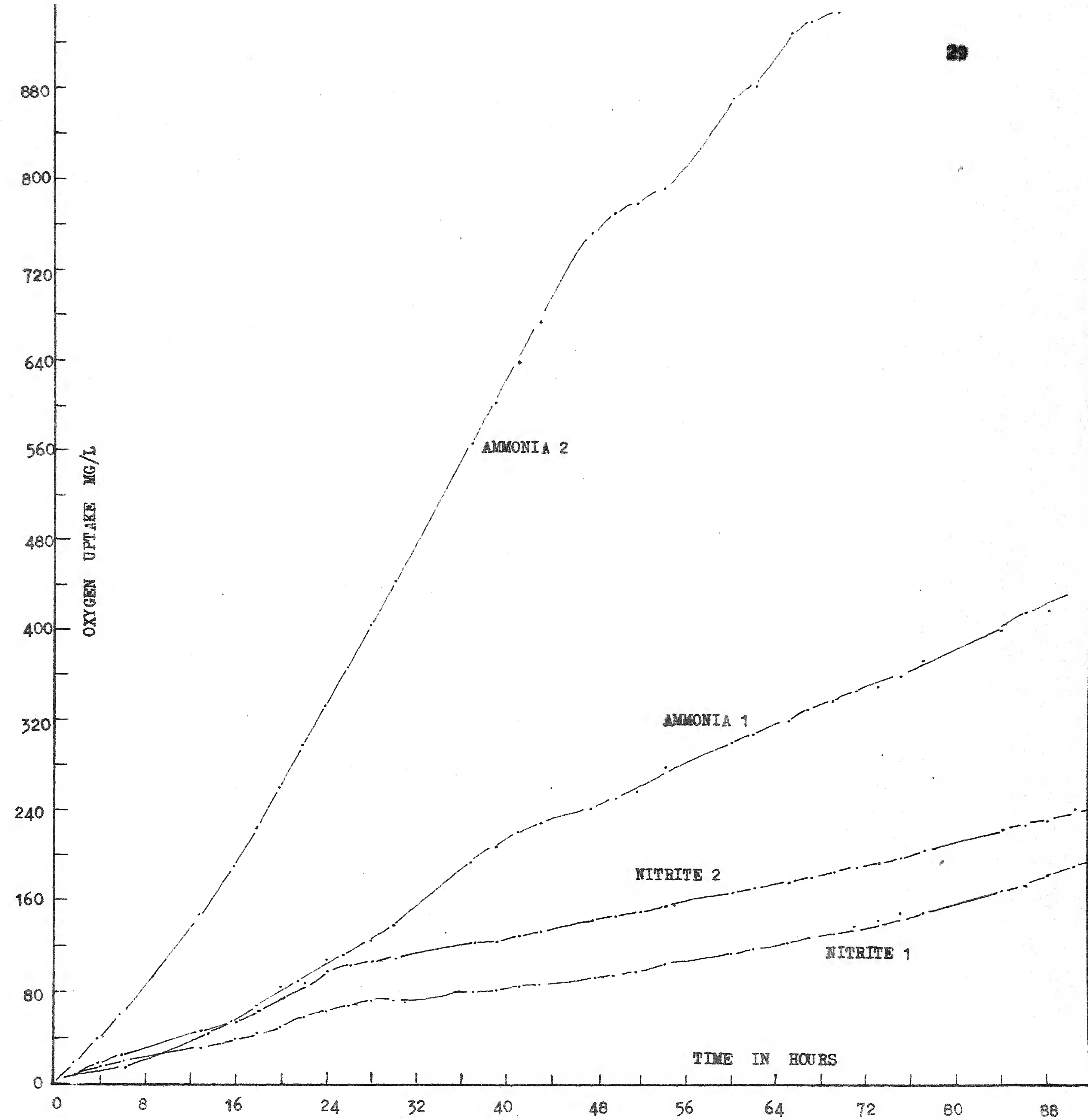

FIG. 7. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 20°C

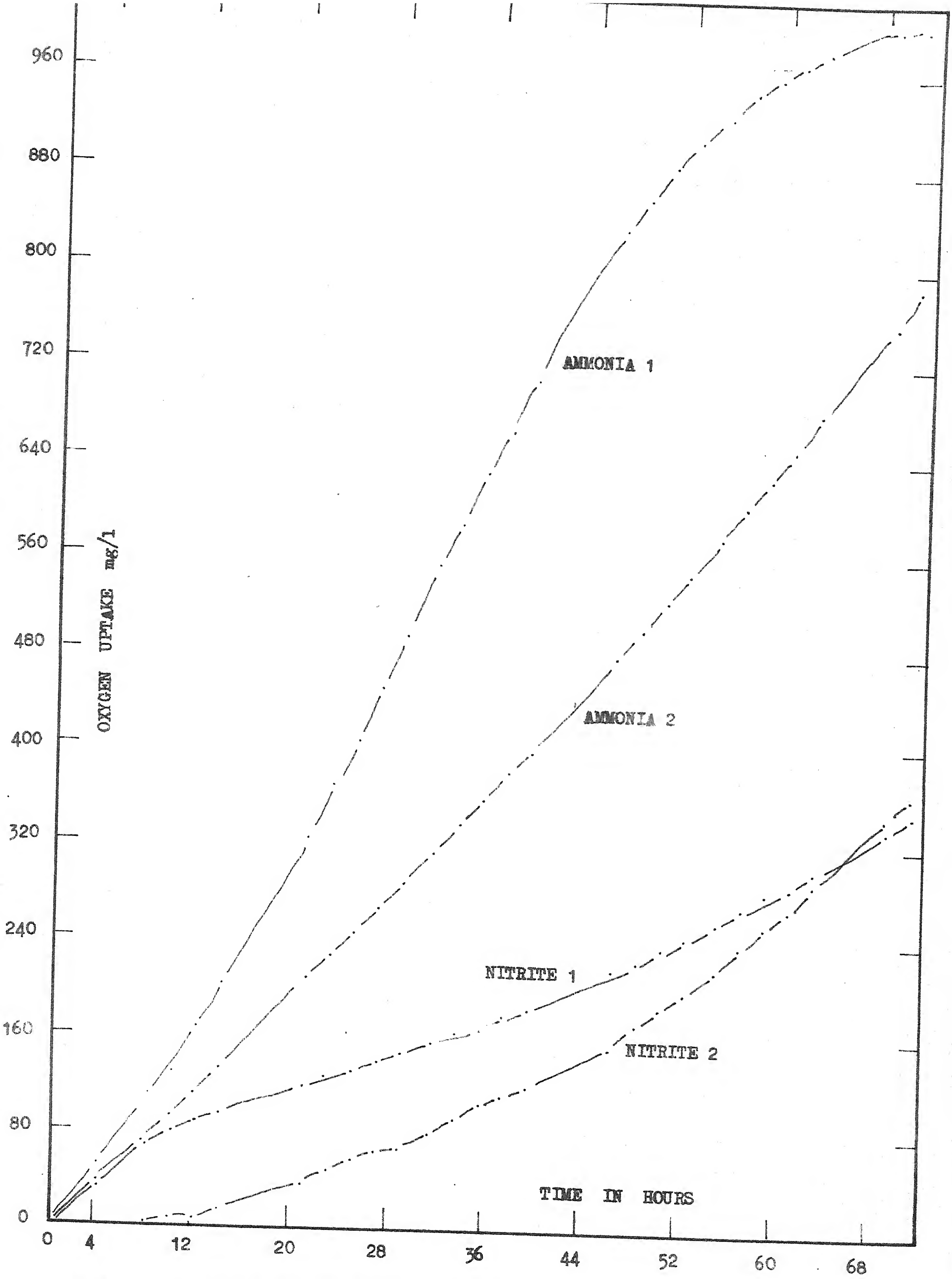

FIG. 8. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXYDATION AT 22°C

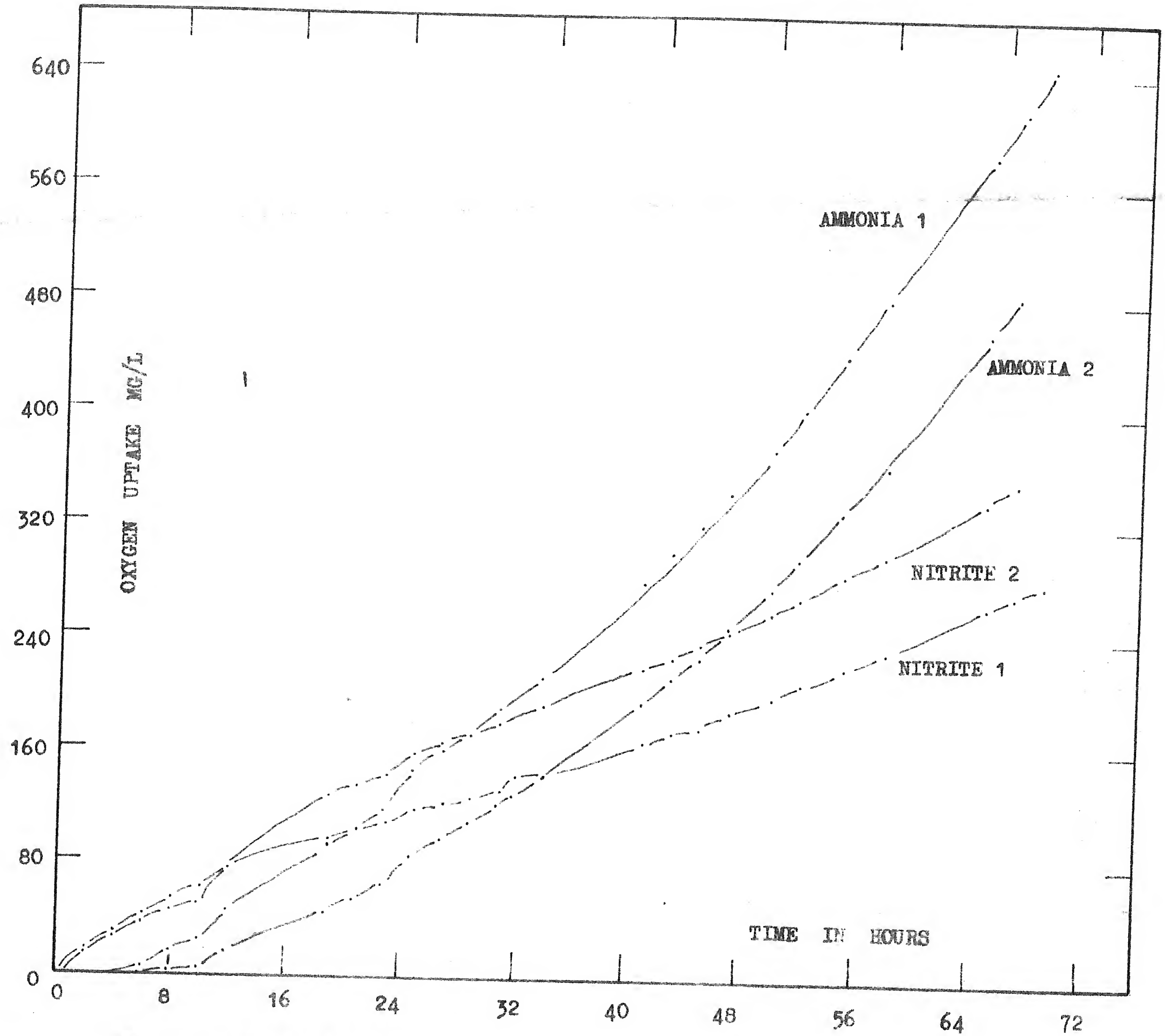

FIG. 9. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 25°C

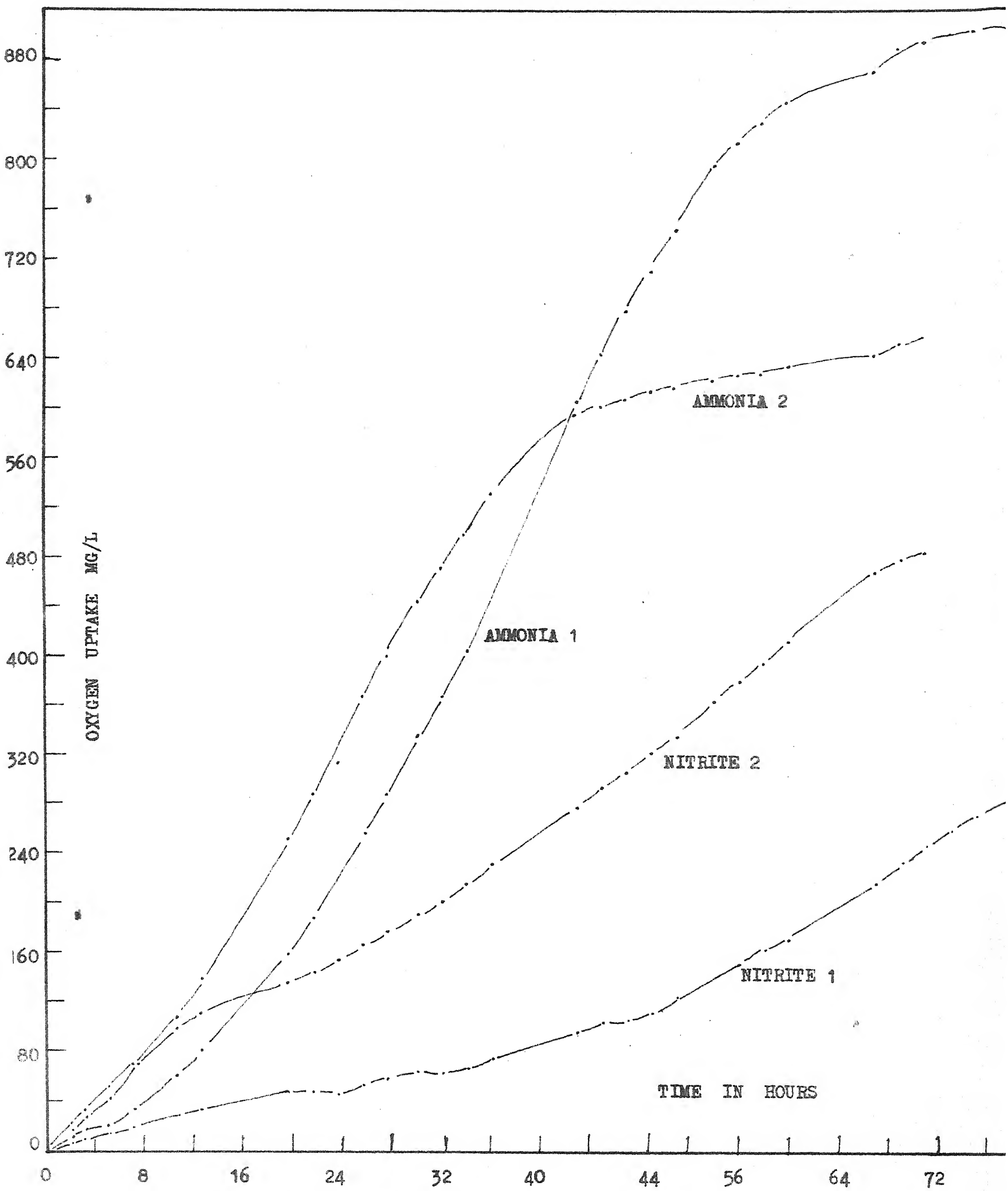

FIG. 10. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 28°C

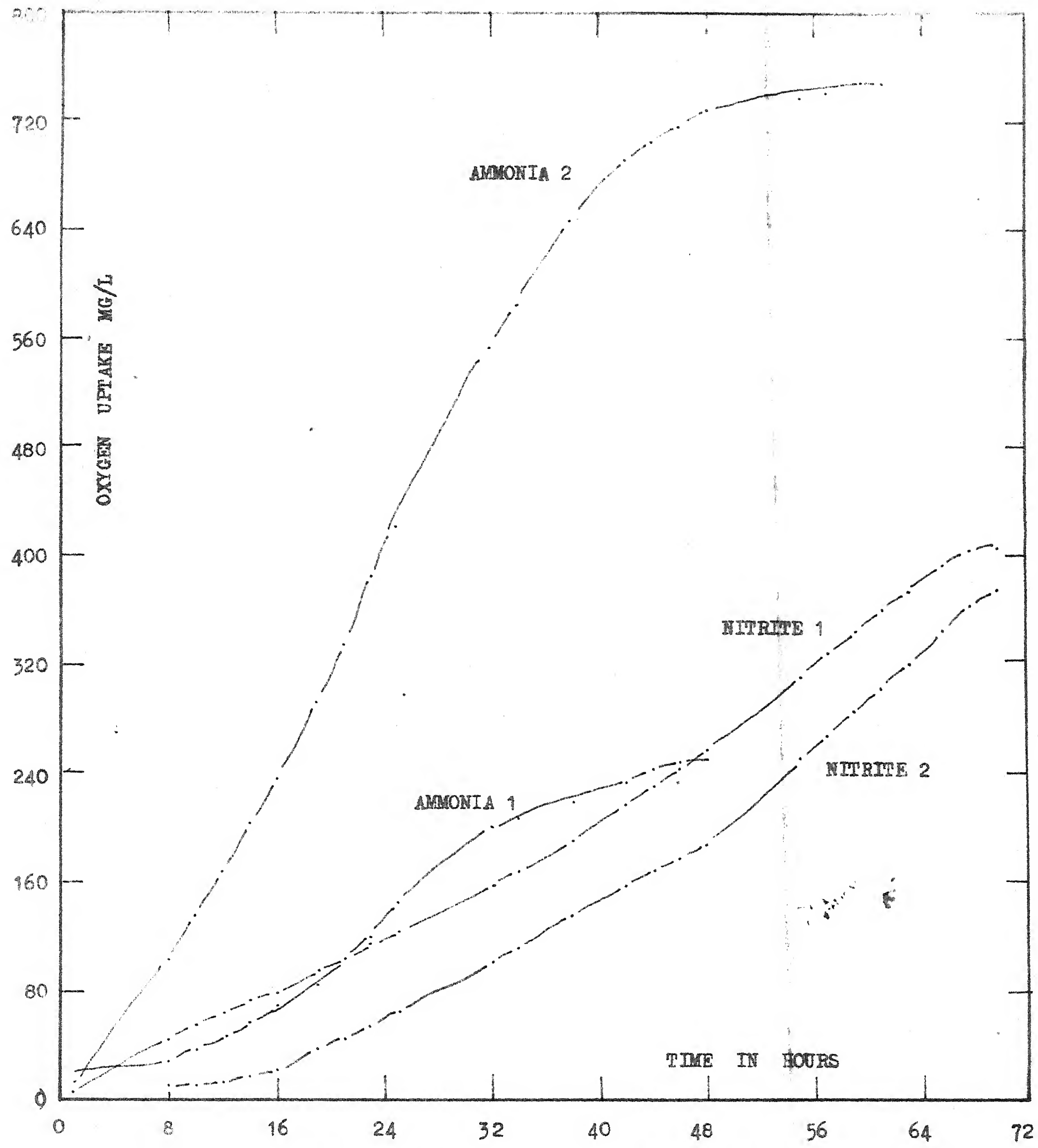

FIG. 11. OXYGEN UPTAKE FOR OXIDATION OF AMMONIA AND NITRITE AT 32 °C

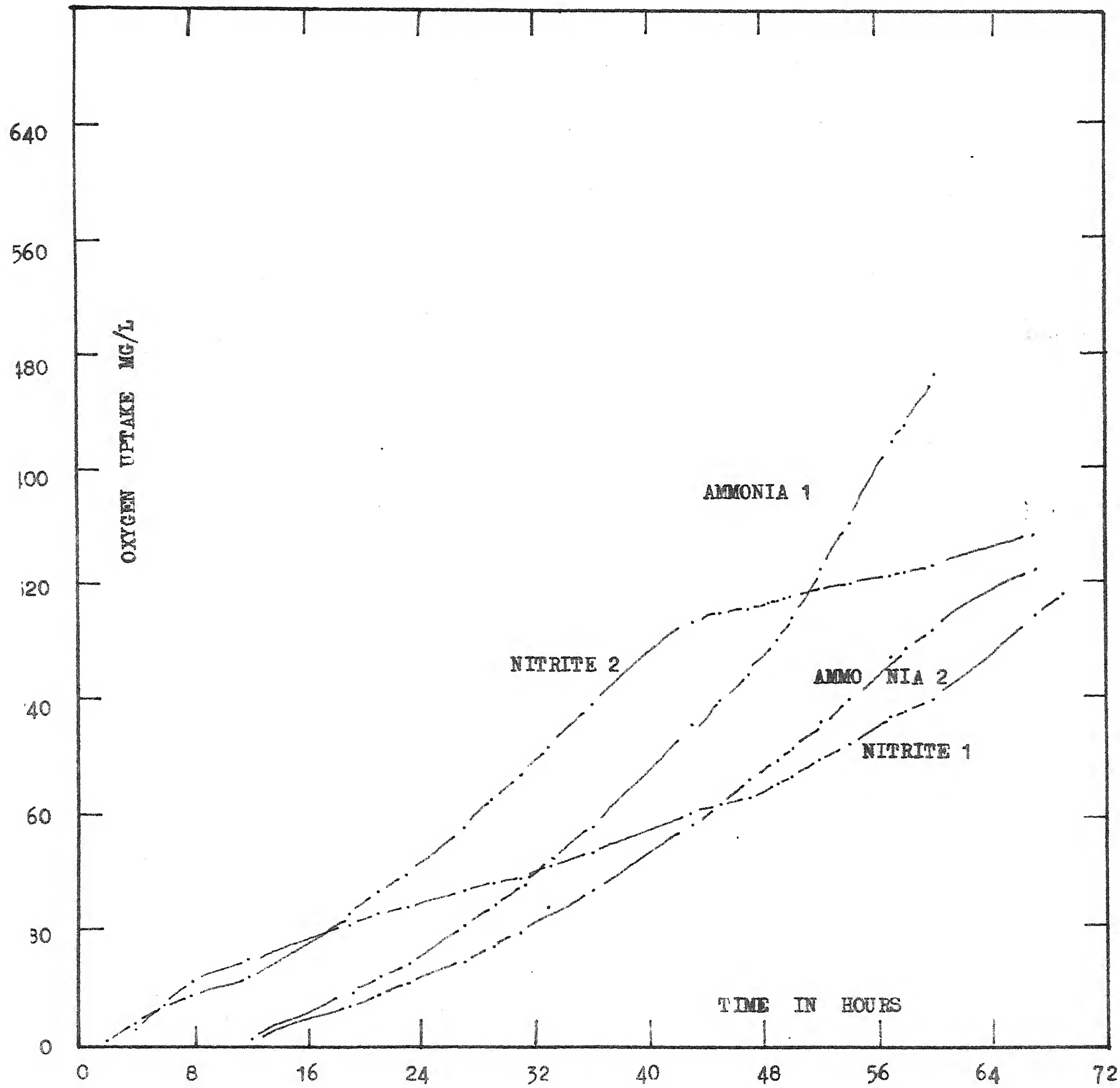

FIG. 12. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 34 °C

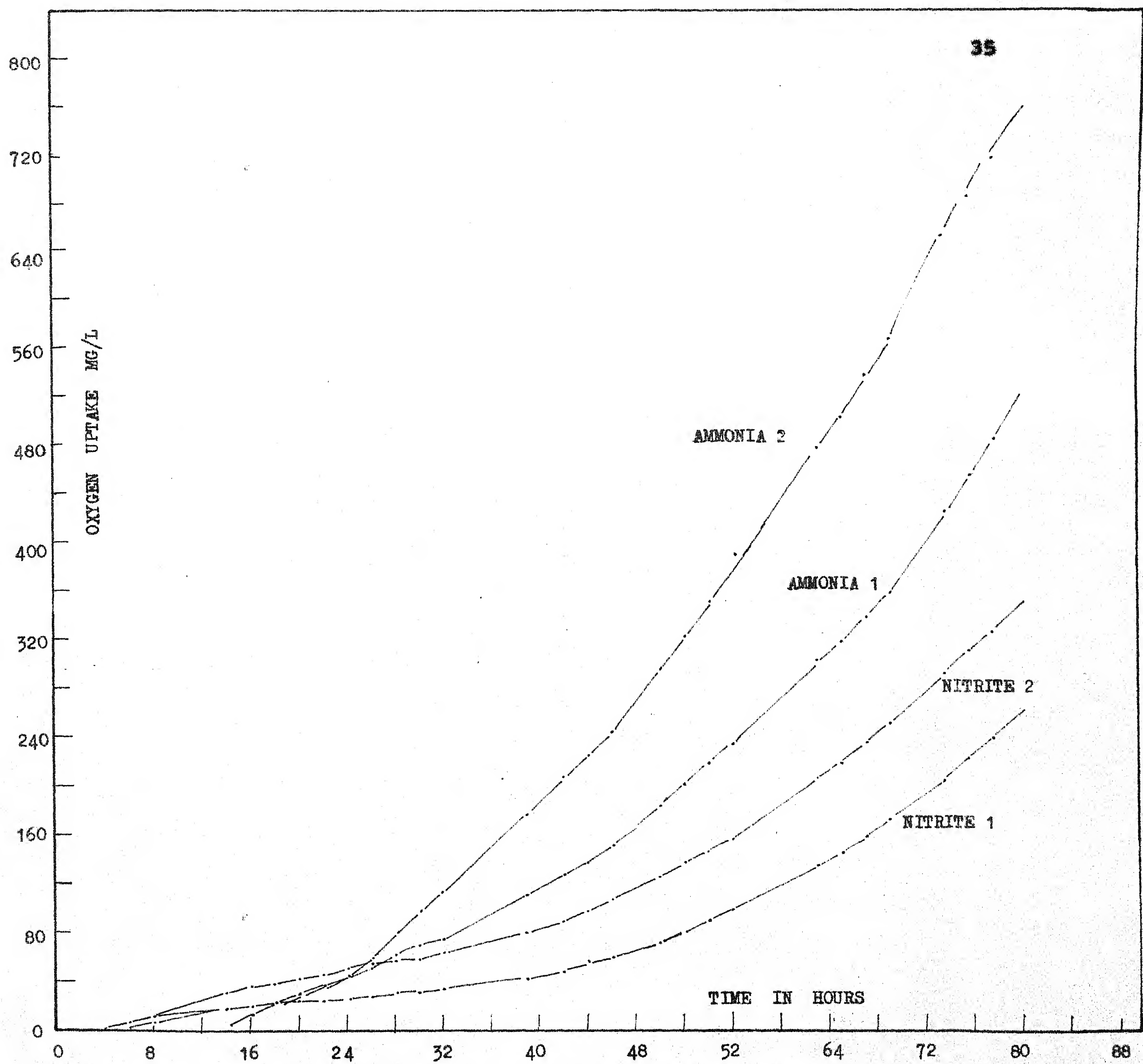

FIG. 13. OXYGEN UPTAKE FOR OXIDATION OF AMMONIA AND NITRITE AT 36°C

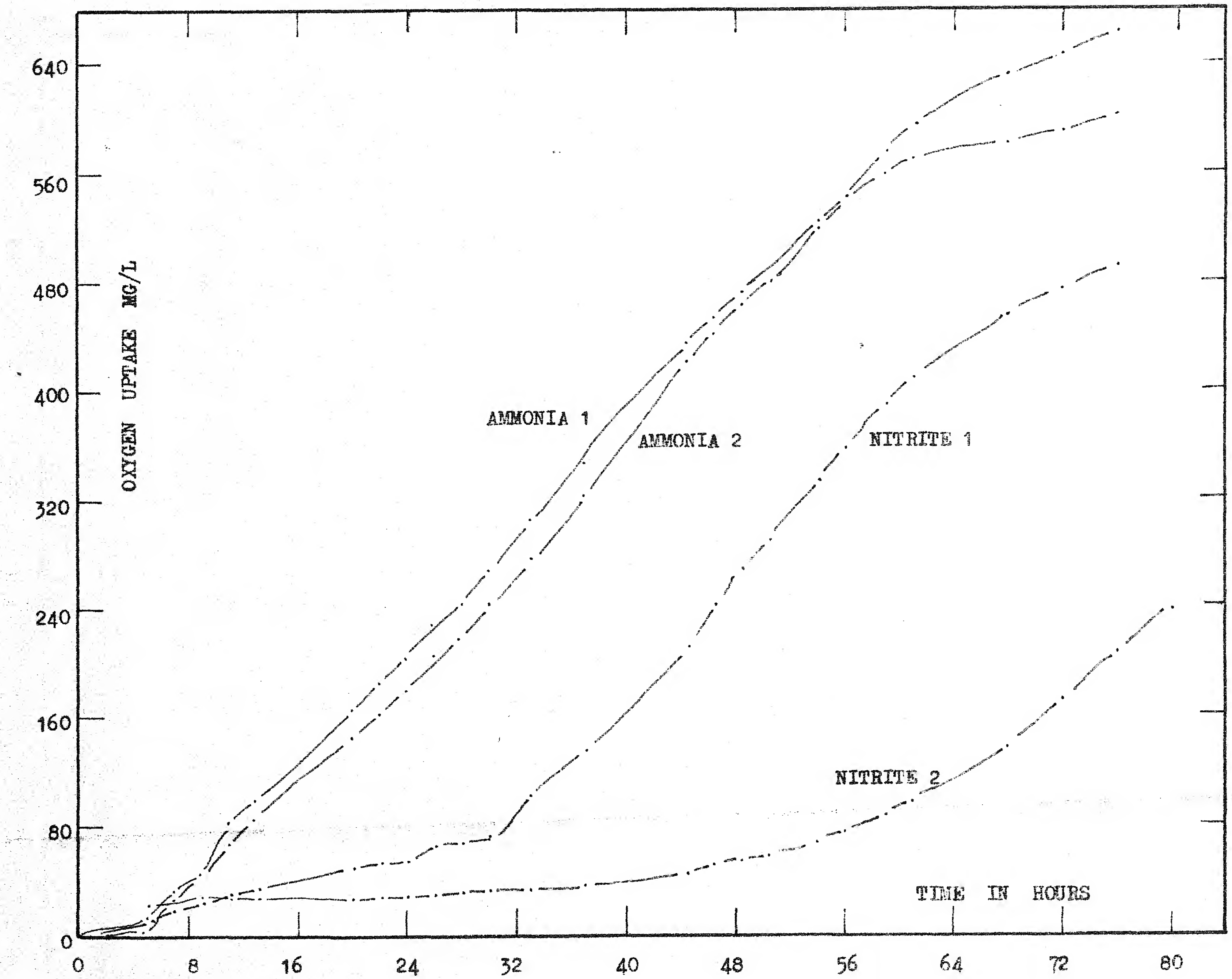

FIG. 14. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 38 oC

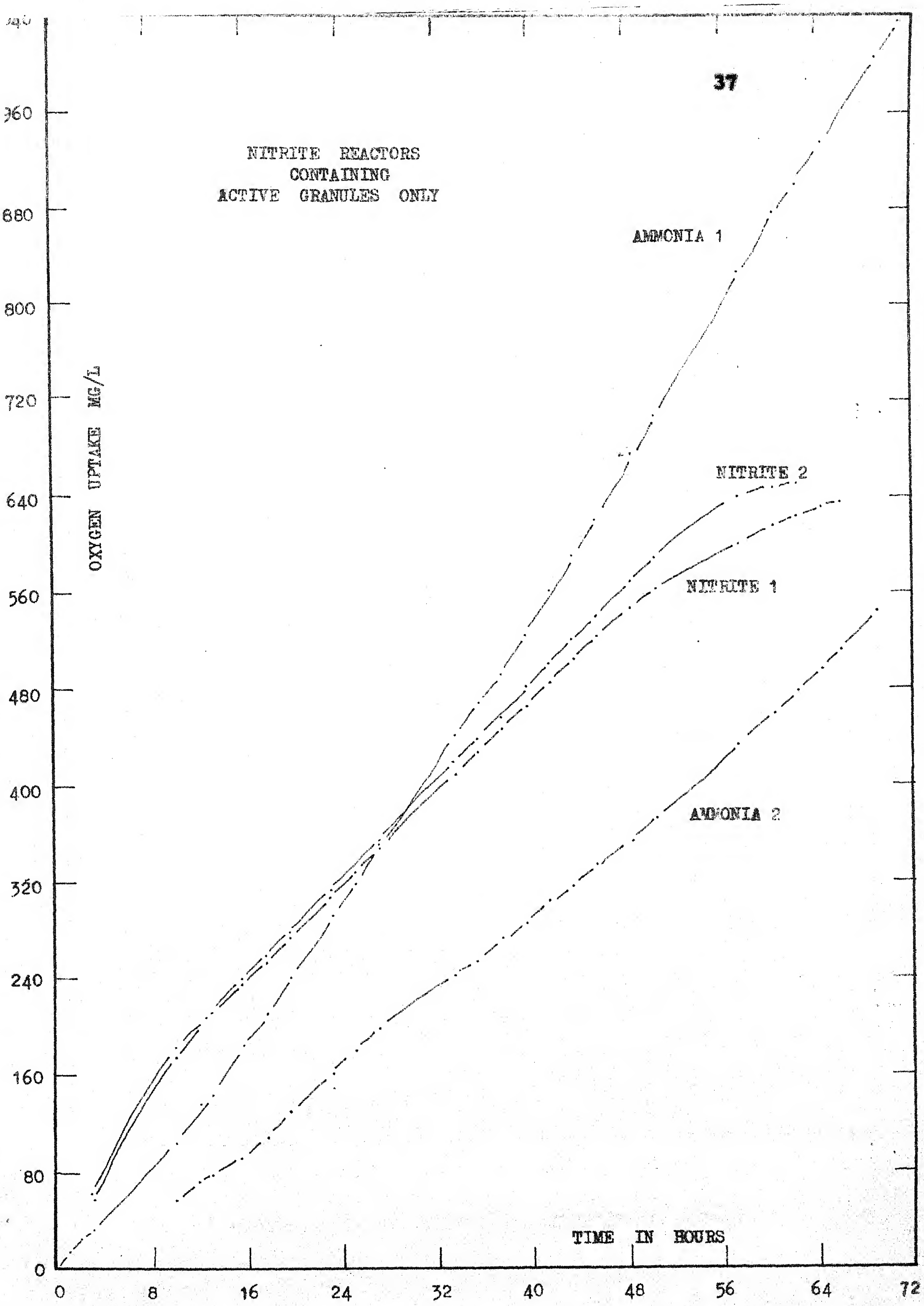

FIG. 15. OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 40 °C

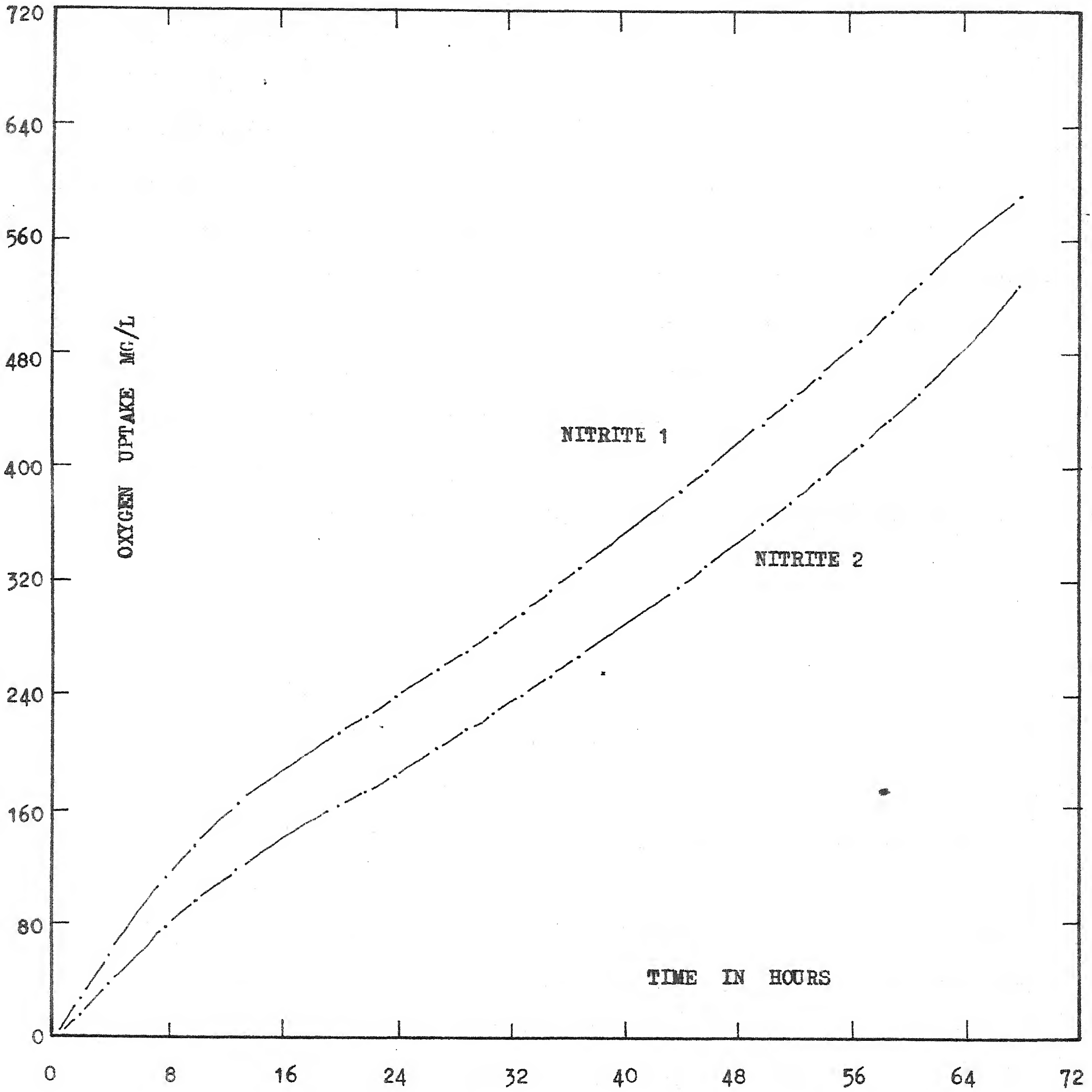

FIG. 16. OXYGEN UPTAKE FOR NITRITE OXIDATION AT 41.5 °C

Fig. 18 shows how growth constant varies with temperature for Nitrosomonas as well as Nitrobacter. In the case of Nitrosomonas growth constant μ_m increased from 0.255 day^{-1} to 0.903 day^{-1} in the temperature range of 20°C to 33°C and then dropped to 0.12 day^{-1} at 40°C. On the other hand, growth constant of Nitrobacter increased from 0.133 day^{-1} to 1.14 day^{-1} in the range of 20°C to about 38°C, and then decreased to 0.42 day^{-1} at 41.5°C. Knowles et al. (18), too, studied the growth of nitrifying bacteria but comparatively at low temperatures (about 8°C to 23°C). In general, their values are higher than those obtained in this work; in the above range of temperature, it increased from 0.2 to 1.02 for Nitrosomonas, and from 0.5 to 1.2 for Nitrobacter.

Knowles et al fitted their data to an equation of the type,

$$\log_{10} \mu = a\ T - b \qquad \ldots \qquad \ldots \qquad (11)$$

where T is in °C

and a, b are constants.

The present data according to this equation for Nitrosomonas are shown in Fig. 19. The best fit follows an equation,

$$\log_{10} \mu_m = 0.0476\ T - 1.545 \qquad \ldots \qquad (12)$$

giving about 11.6 percent increase in μ_m per degree centigrade, as compared to Kowles' 9.5 percent.

And for Nitrobacter it will be, Fig. 20,

$$\log_{10} \mu_m = 0.0495\ T - 1.86 \qquad \ldots \qquad (13)$$

giving about 12.0 percent increase in μ_m per degree centigrade, as compared to Knowles 5.9 percent.

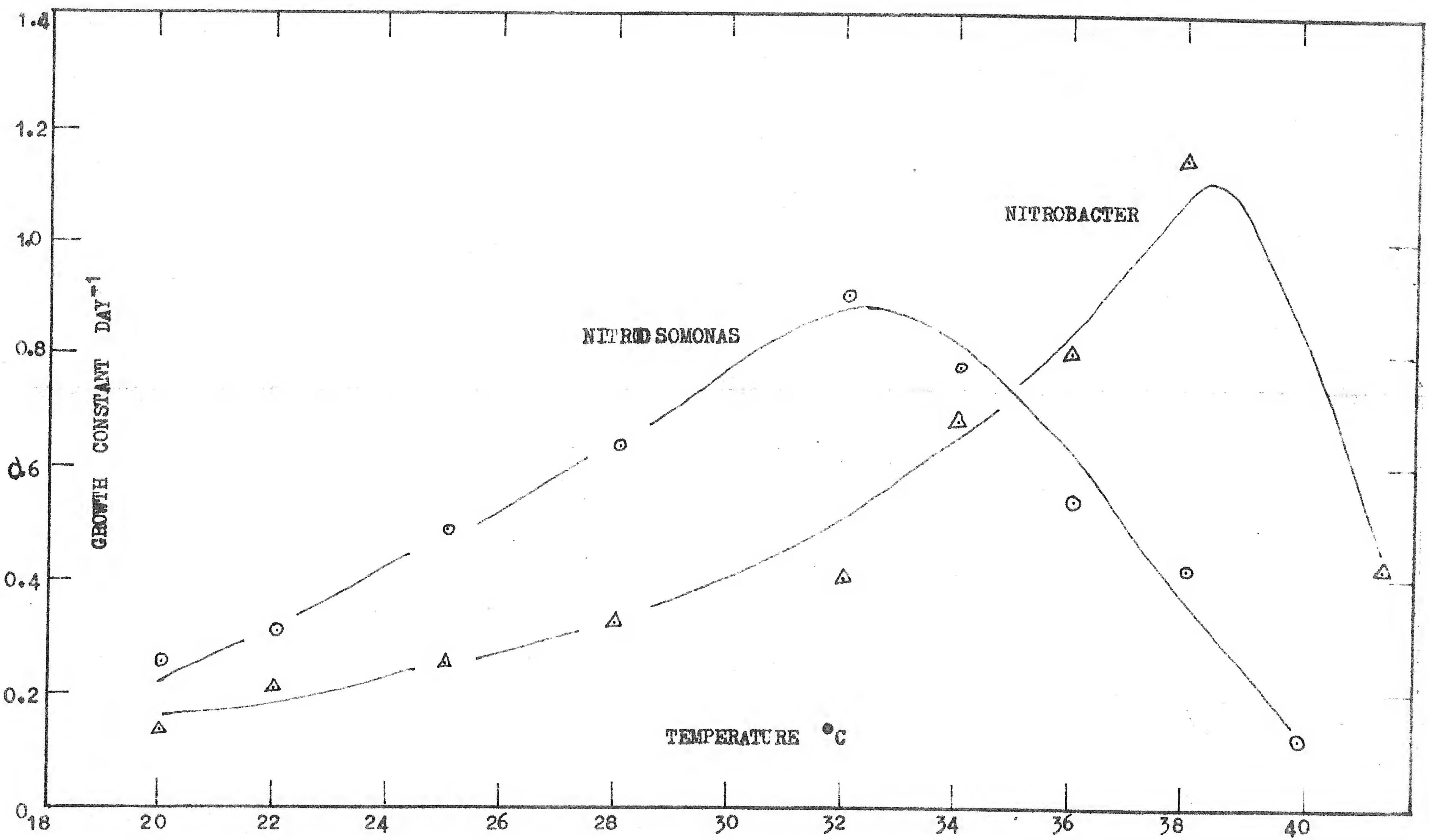

FIG. 18. VARIATION OF GROWTH CONSTANT WITH TEMPERATURE

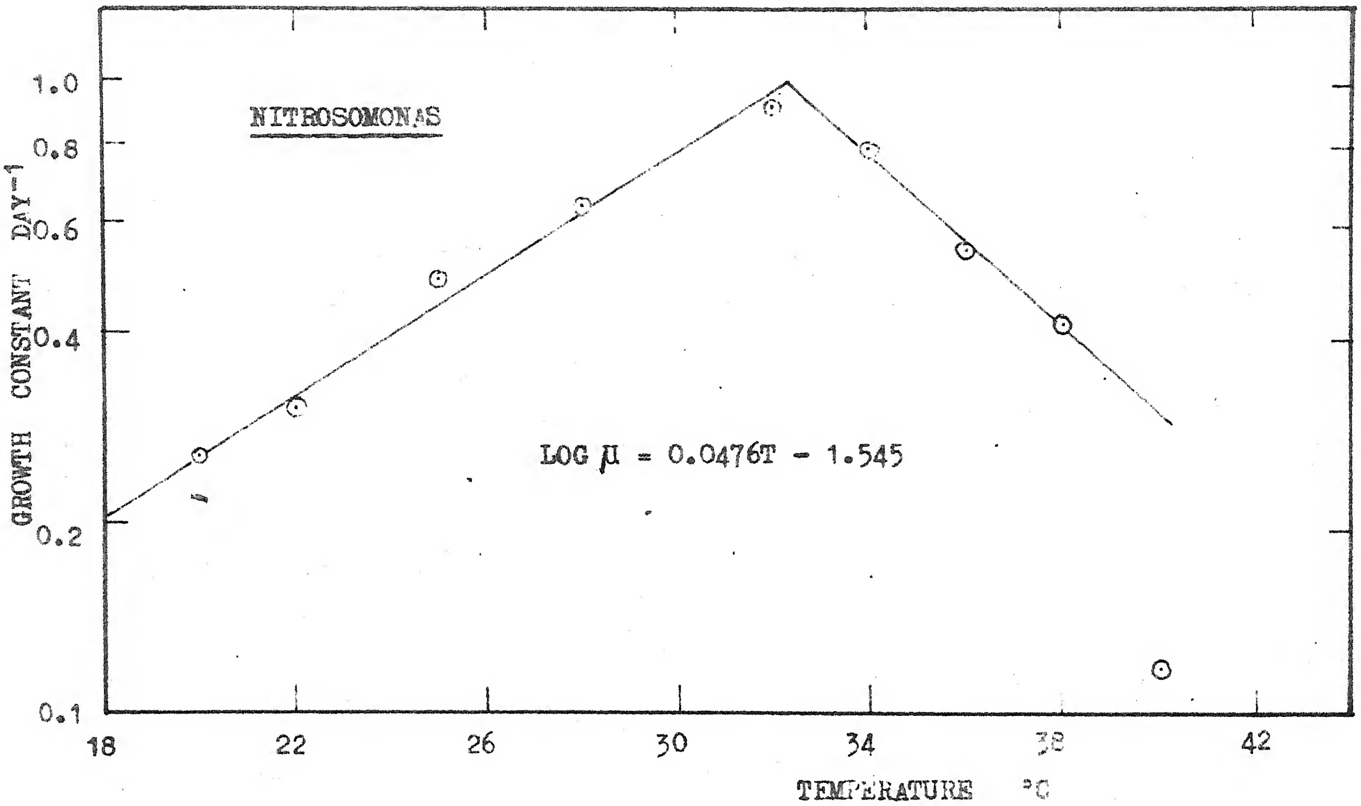

FIG. 19. VARIATION OF GROWTH CONSTANT WITH TEMPERATURE

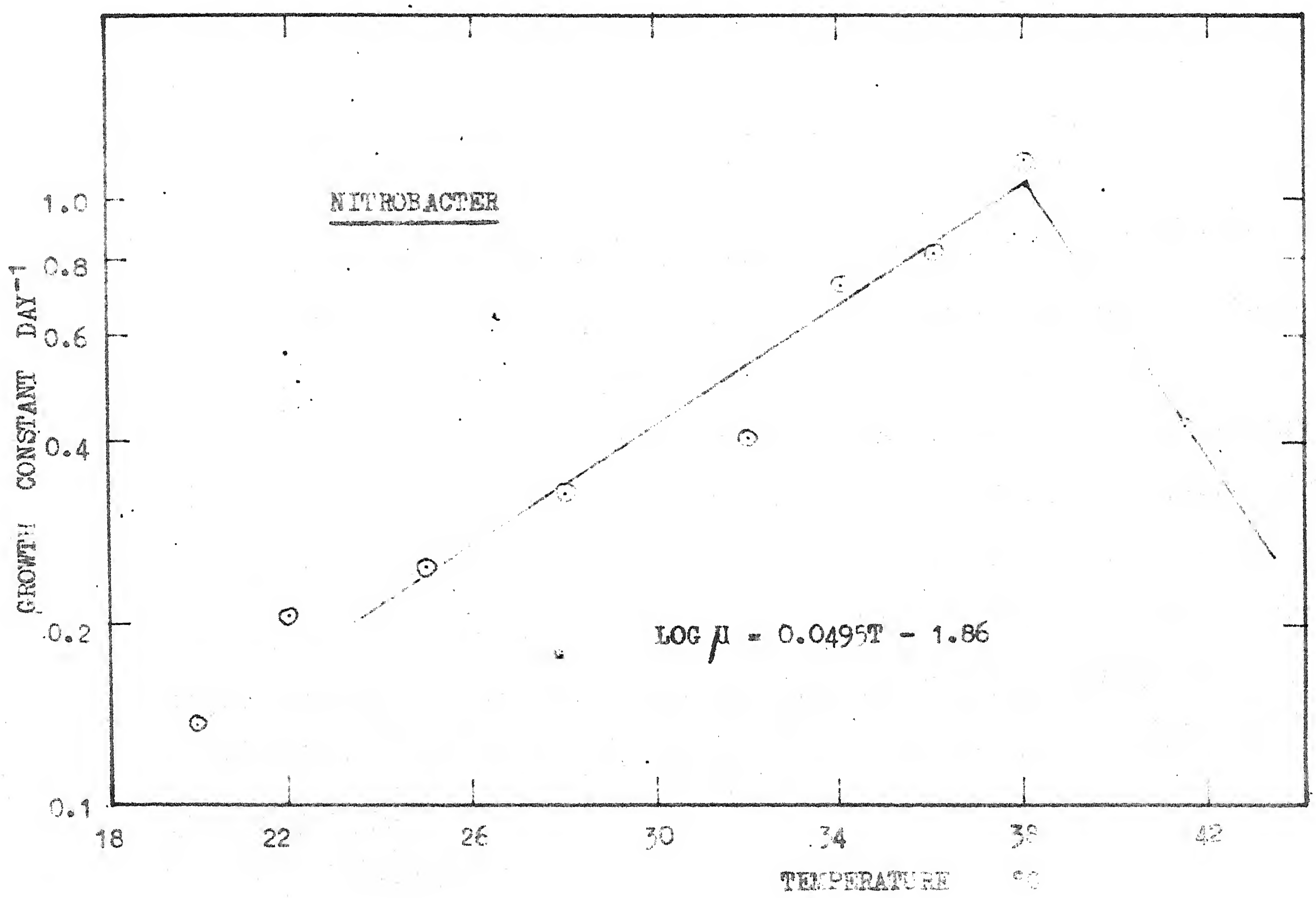

FIG. 20. VARIATION OF GROWTH CONSTANT WITH TEMPERATURE

The a,b values as found by Knowles are 0.0413 and 0.944 for Nitrosomonas, and 0.0255 and 0.492 for Nitrobacter.

Equations (12) and (13) can be converted to another form used by Phelps, (29). For Nitrosomonas it becomes

$$\frac{\mu_1}{\mu_2} = 1.116^{t_1 - t_2} \quad \ldots \quad \ldots \quad (14)$$

and for Nitrobacter,

$$\frac{\mu_1}{\mu_2} = 1.12^{t_1 - t_2} \quad \ldots \quad \ldots \quad (15)$$

Equations (14) and (15) are comparable to those used by Gotaas (30) for deoxygenation constant K of sewage,

$$\frac{K_1}{K_2} = \theta^{t_1 - t_2} \quad \ldots \quad \ldots \quad (16)$$

where θ is called temperature coefficient, and t is temperature in $^{\circ}$C.

He obtained temperature coefficient ranging from 0.9672 to 1.1086.

Carpenter et al (31) also used similar equation while studying the effect of temperature on treatment of paper mill waste. He found θ as 1.016.

However it should be realised that the above equations are valid for a limited range of temperature only. Equation (12) and (14) are valid upto about 32°C, and equation (13) and (15) are upto 38°C. At temperatures above these values growth rate falls instead of rising which is typical of any biological process.

<u>Arrhenius Plot:</u>

Logarithm of growth constant has been plotted against

inverse of temperature in absolute degrees, in Fig. 21. Both for Nitrosomonas and Nitrobacter, the curves are linear over a lower range of temperature. Thus growth constant temperature relationship follows Arrhenius Law, the equation being

$$\mu = \mu_1 e^{-E_1/RT} \qquad \ldots \qquad \ldots \qquad (17)$$

However with further increase in temperature, the above equation apparently does not hold good, and growth constant falls off in the negative side with increasing temperature. Enzyme inactivation and denaturation of cell material are known to be some of the causes of this lowering of growth. Hinshelwood has included a second term in the above equation to take care of this effect. The complete equation takes the form,

$$\mu = \mu_1 e^{-E_1/RT} - \mu_2 e^{-E_2/RT} \qquad \ldots \qquad \ldots \qquad (18)$$

The temperature characteristic E_1 as determined from the slope of the initial portion of the curves is 17,150 cal/mole for the oxidation of ammonia by Nitrosomonas and is 17,600 cals/mole for the oxidation of nitrite by Nitrobacter.

Ingraham (14) found a value of 14,200 cals/mole in the case of E.Coli and for the same bacterium Johnson and Lewin (13) found 15,000 cals/mole.

It should be realised that the second term in the above equation represents a purely chemical reaction, viz., heat coagulation of proteins. Generally E_2 values are quite high. Depending on the nature of protein it varies from 60,000 to 130,000 cals/mole (8). For the cell material of Nitrosomonas and Nitrobacter these values are found to be around 88,000 cals/mole.

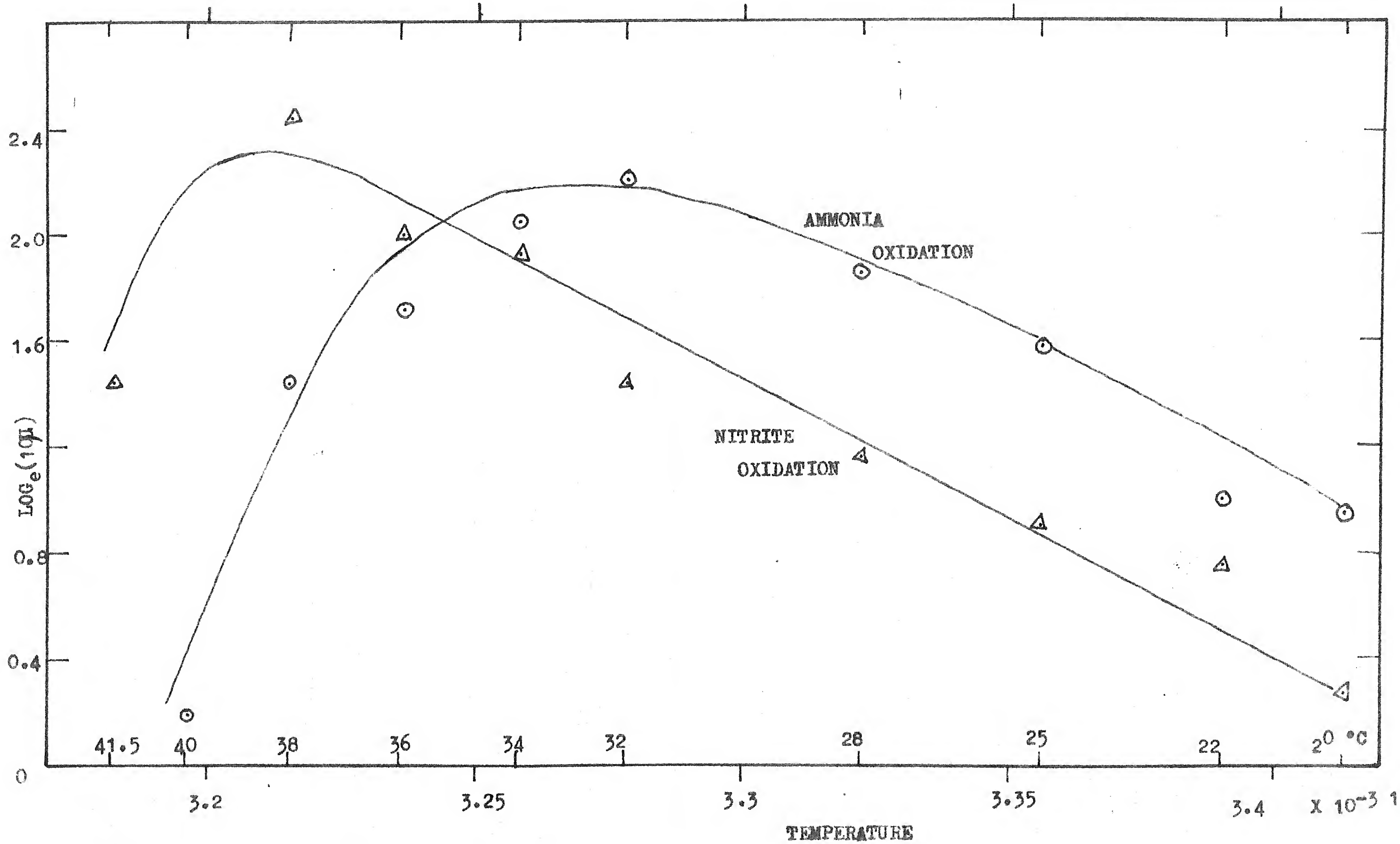

FIG. 21. ARRHENIUS PLOT OF GROWTH RATES FOR NITRIFYING BACTERIA

Since E_2 is much larger compared to E_1 (more than about five times), the second term in the above equation is negligible as compared to the first term in the low range of temperature. And as a result we get a straight line in the plot of log μ against 1/T °K. When the temperature is increased further, the second term becomes comparable to first one, and the curves of ammonia oxidation and nitrite oxidation pass through a temporary stationary phase. With still further increase in temperature, the second term outweighs the first one, and since E_2 is quite high the curves drop with temperature rapidly.

Generation Time

Generation time and growth constant are related by

$$t_g = \log_e 2/\mu \quad \ldots \quad \ldots \quad (19)$$

Generation times have been calculated from growth constants at various temperatures, and plotted in Fig. 22.

Generation time of Nitrosomonas decreased upto 32°C to a value of 0.72 days and then increased considerably to a value of 4.8 days at 40°C. That of Nitrobacter decreased from a high value of 4.9 days to a value of 0.76 days at 38°C and then started increasing.

The optimum temperature for the growth of Nitrosomonas seems to be around 34°C and of Nitrobacter around 38°C.

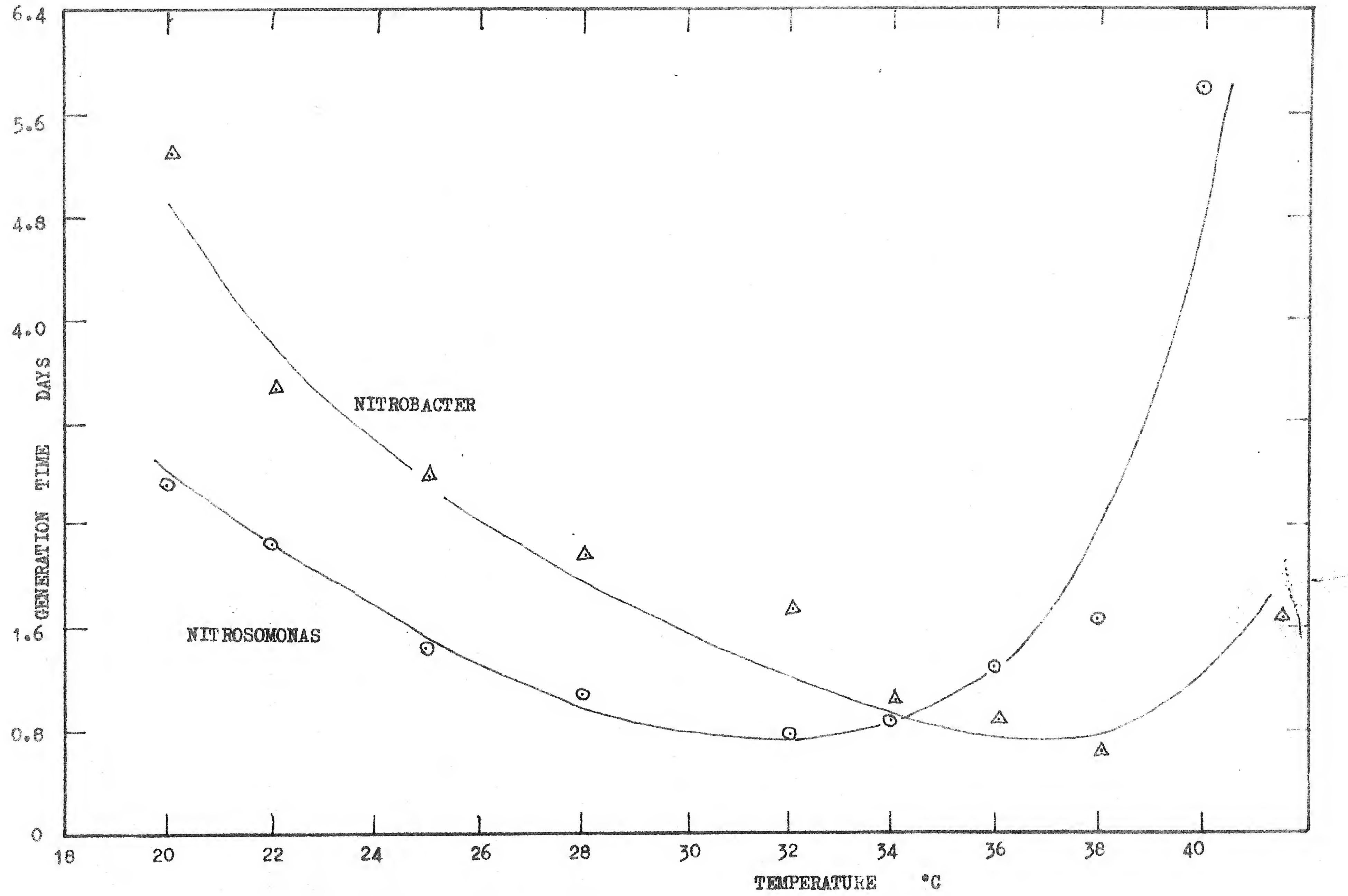

FIG. 22. GENERATION TIME OF NITRIFYING BACTERIA AT DIFFERENT TEMPERATURES

5. CONCLUSIONS

Based on the above discussions following conclusions can be drawn.

1. Growths of Nitrosomonas and Nitrobacter are affected considerably by variation of temperature.

2. Growth constant μ_m varies from 0.1195 day^{-1} in the minimum to 0.903 day^{-1} in the maximum for Nitrosomonas and from 0.133 day^{-1} to 1.14 day^{-1} for Nitrobacter.

3. Optimum temperature for growth of Nitrosomonas is around 34°C and that of Nitrobacter is around 38°C. On both higher and lower sides of these optima, growth is affected adversely. The rate of this effect at increasing temperature is much more than at decreasing temperature.

4. Growth of Nitrosomonas can be increased about 11.6 percent per degree rise in temperature, and growth of Nitrobacter about 12.0 percent, provided the temperatures are below optima.

5. Temperature characteristic (or activation energy) for the oxidation of ammonia by Nitrosomonas seems to be 17,150 cals/mole and that for the oxidation of nitrite by Nitrobacter to be 17,600 cals/mole.

6. Generation time of Nitrosomonas varied from 0.72 days to a maximum of 5.8 days, and that of Nitrobacter varied from 0.72 days to 5.3 days.

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APPENDIX A

TABLE 7

FLASK CONSTANTS AT DIFFERENT TEMPERATURES

Dry Weight of Flasks (gms.)	FLASK CONSTANTS (µl/mm) AT									
	20°C	22°C	25°C	28°C	32°C	34°C	36°C	38°C	40°C	41.5°C
29.634	1.24	1.23	1.22	1.206	1.19	1.18	1.17	1.167	1.159	1.153
28.684	1.432	1.43	1.409	1.39	1.375	1.365	1.356	1.347	1.339	1.332
27.602	1.368	1.36	1.3455	1.33	1.31	1.30	1.295	1.285	1.278	1.272
21.1903	1.868	1.82	1.8365	1.77	1.792	1.781	1.769	1.757	1.746	1.737
20.637	1.819	1.81	1.7875	1.715	1.746	1.734	1.722	1.717	1.7	1.69
19.532	1.761	1.75	1.7315	1.715	1.69	1.682	1.67	1.659	1.648	1.638
28.3805	1.477	1.46	1.452	1.435	1.417	1.405	1.395	1.391	1.381	1.372
22.5836	1.758	1.746	1.729	1.70	1.68	1.675	1.664	1.646	1.645	1.636
21.0795	1.852	1.84	1.821	1.798	1.772	1.766	1.755	1.75	1.733	1.724
20.1858	1.817	1.805	1.787	1.762	1.736	1.732	1.72	1.714	1.698	1.688

TABLE 8

SOLUBILITY OF OXYGEN (27)

Temperature °C	Solubility of Oxygen, α, ml. oxygen dissolved per ml of water when gas is at 1 atm.
0	.04872
10	.03793
15	.03441
20	.03091
25	.02822
30	.02612
35	.0245
40	.0230

APPENDIX B

TABLE 9

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 20°C

Time hrs	NH_3 Rector I a	b	c	d	e	NH_3 Rector II a	b	c	d	e	NO_2 Rector I a	b	c	d	e	NO_2 Rector II a	b	c	d	e	Thermobarometer a	b
6	229					245					184					131					178	
8	219	-10	- 3			220	- 25	-18	18	16	171	-13	- 6	6	6	117	-14	- 7	7	7	171	- 7
10	202	-17	+ 1			178	- 42	-24	42	37	148	-23	- 5	11	11	9	-27	- 9	16	16	153	-18
12	197	- 5	- 8	8	11	157	- 21	-24	66	59	145	- 2	- 5	16	16	86/183	- 4	- 7	23	23	156	+ 3
19	161	-36	-24	32	40	45/237	-112	-100	166	147	122/179	-24	-12	28	29	150	-33	-21	44	43	144	-12
22	133	-28	-10	42	53	171	- 46	-48	214	190	153	-26	- 8	36	37	122	-28	-10	54	53	126	-18
24	131	- 2	-11	53	67	140	- 31	-40	254	225	158	+ 5	- 4	40	41	121	- 1	-10	64	63	135	+ 9
26	132	+ 1	-12	65	82	112	- 28	-41	295	262	164	+ 6	- 7	47	48	123	+ 2	-11	75	73	148	+13
28	131	- 1	- 5	7	88	75/251	- 37	-41	336	298	159	- 5	- 9	56	574	116	- 7	-11	86	84	152	+ 4
30	118	-13	-10	80	107	208	- 43	-40	376	333	152	- 7	- 4	60	615	99	-17	-14	100	98	145	- 3
32	94	-24	- 9	89	112	153	- 55	-40	416	368	132	-20	- 5	65	67	79	-20	- 5	105	103	134	-15
34	72	-22	-10	99	125	101	- 52	-40	456	404	115	-17	- 5	70	72	63	-16	- 4	109	107	122	-12
36	57/192	-15	-11	110	139	57/242	- 44	-40	496	440	111/172	- 4	0	70	72	56/17	- 7	- 3	112	110	118	- 4
43	141	-51	-47	157	198	94	-148	-144	64	567	160	-12	- 8	78	80	151	-19	-15	127	124	114	- 4
45	123	-18	- 9	166	209	45/243	- 49	-48	68	603	149	-11	- 2	80	82	14	-11	- 2	129	126	105	- 9
47	112	-11	-10	176	222	200	- 43	-42	722	640	144	- 5	- 4	84	86	134	- 6	- 5	134	131	104	- 1
49	123	+11	- 7	183	230	178	- 22	-40	762	676	160	+16	- 2	86	88	148	+16	- 4	138	135	122	+18
53½	126	+ 3	-11	194	244	102	- 76	-90	852	755	168	+ 8	- 6	92	94	152	+ 4	-10	148	145	136	+14

55½	107	-19	- 8	202	252	71	-31	-20	872	773	155	-13	- 2	94	96	138	-14	- 3	151	148	125	-11
57½	83	-24	- 4	206	259	41	-30	-10	882	782	131	-24	- 4	98	100	114	-24	- 4	155	152	105	-20
60½	74/196	- 9	-17	223	282	35/225	- 6	-14	896	795	133	+ 2	- 6	104	107	116	+ 2	- 6	161	158	113	+ 8
66½	177	-19	-18	241	303	132	-93	-92	988	876	122	-11	-10	116	117	101	-15	-14	175	171	112	- 1
68½	155	-22	- 6	247	311	101	-31	-15	1003	886	102	-20	- 4	118	121	81	-20	- 4	179	175	96	-16
71½	155	0	-10	257	323	60	-41	-51	1054	934	106	+ 4	- 6	124	127	86	+ 5	- 5	184	180	106	+10
73½	167	+12	- 9	266	335	70	+10	-11	1065	944	123	+17	- 4	128	132	100	+16	- 5	189	185	127	+21
75½	175	+ 8	- 6	272	342	77	+ 7	- 7	1072	950	133	+10	- 4	132	135	109	+ 9	- 5	194	190	141	+14
77½	170	- 5	- 7	279	351						131	- 2	- 4	136	139	107	- 2	- 4	198	194	143	+ 2
79½	156	-14	- 3	282	355			Stopped			116	-15	- 4	140	144	90	-17	- 6	204	199	132	-11
81½	140	-16	- 8	290	365						103	-13	- 5	145	149	77	-13	- 5	209	204	124	- 8
83½	132	- 8	-11	301	379						99/155	- 4	- 7	152	156	74/187	- 3	- 6	215	210	127	+ 3
90½	97	-35	-21	322	405						121	-34	-20	172	176	153	-34	+20	235	230	113	-14
92½	67	-30	-13	335	422						100	-21	- 4	176	180	131	-22	- 5	240	234	96	-17
94½	56	-11	- 3	338	425						84	-16	- 8	184	189	119	-12	- 4	244	238	88	- 8
97	60	+13	-12	350	440						99	+15	-10	194	199	133	+14	-11	255	249	113	+25
99	71										100	+ 1	- 6	200	205	135	+ 2	- 5	260	254	120	+ 7

Flask constants	1.7613	1.24	1.4323	1.3683
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	570	390	-	-
Final NO_2	110	260	520	480
Final NO_3	20	50	180	220

TABLE 10

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 22°C

Time hrs.	NH_3 Reactor I a	b	c	d	e	NH_3 Reactor II a	b	c	d	e	NO_2 Reactor I a	b	c	d	e	NO_2 Reactor II a	b	c	d	e	Thermo barometer	
9	288					252					240					233					195	
13	254/193	- 34	- 50	50	51	240/186	-12	-28	26	35	224/163	-16	-32	32	31	285/151	+52	+36			211/15	+1
17	152	- 41	- 54	104	106	171	-15	-28	56	70	140	-23	-36	68	66	162	+12	- 1	1	1	163	+1
19	125	- 27	- 20	124	126	146	-23	-16	72	90	121	-15	-12	60	78	148	-14	- 7	8	7	156	-
21	73	- 52	- 36	160	166	116	-32	-16	86	110	97	-24	- 8	68	86	134	-14	+ 2	6	5	14	-1
23	38/291	- 35	- 36	196	199	101/162	-15	-16	104	130	90/156	- 7	- 8	56	93	125/221	- 9	-10	16	14	141/175	+
30	170	-121	-120	316	321	101	-61	-60	164	205	131	-25	-24	120	117	194	-27	-26	42	37	174	-
32	112	- 58	-44	360	366	71	-30	-16	180	225	109	-22	- 8	128	125	168	-26	-12	54	48	160	-1
34	69	- 43	-36	396	403	48	-23	-16	196	245	96	-13	- 6	134	130	150	-18	-11	65	57	153	-
36	32/283	- 37	-44	440	447	39/174	- 9	-16	212	265	95/185	-1	- 8	142	138	149/197	- 1	- 8	73	64	160/150	+
38	251	- 32	-44	484	492	170	- 4	-16	228	285	190	+5	- 7	149	146	207	+10	- 2	75	66	162	+1
40½	194	- 57	-56	540	549	149	-21	-20	248	310	180	-10	- 9	158	154	191	-16	-15	90	70	161	-
42½	153	- 41	-36	576	585	125	-24	-19	267	334	167	-13	- 8	166	162	172	-19	-14	104	92	156	-
44½	104	- 49	-40	616	626	99	-26	-17	284	355	154	-13	- 4	170	165	149	-23	-14	118	104	147	-
46½	63	- 41	-34	650	660	74	-25	-18	302	378	135	-19	-12	188	177	134	-15	- 8	126	111	140	-
48½	24/251	- 39	-42	692	703	63/151	-11	-14	316	395	132/177	- 3	- 6	188	183	124/192	-10	- 7	133	118	143/150	+3

Contd..........

55½	112	−139	−124	816	830	72	−79	−64	380	475	130	−47	−32	220	215	137	−55	−40	173	152	135	−15
57½	78	− 34	− 26	842	855	46	−26	−18	398	498	114	−16	− 8	228	222	108	−29	−21	194	171	127	− 8
59½	60/184	− 18	− 26	868	882	36/176	−10	−18	416	520	110	− 4	−12	240	234	10/188	− 8	−16	210	185	135	+ 8
61½	180	− 4	− 20	888	904	172	− 4	−20	436	545	116	+ 6	−10	250	243	189	+ 1	−15	225	198	166	+16
64	167	− 13	− 22	910	925	157	−15	−24	460	575	113	− 3	−12	262	255	176	−13	−22	247	217	175	+ 9
66	143	− 24	− 20	930	945	131	−26	−22	482	602	95	−18	−14	276	269	157	−19	−23	270	238	171	− 4
68	111	− 32	− 12	942	956	93	−38	−18	500	625	61	−34	−14	290	282	118	−39	−19	289	254	151	−20
70	90	− 21	− 12	954	968	64	−29	−20	520	650	46	−15	− 6	296	286	96	−22	−13	302	266	142	− 9
72	83/142	− 7	− 10	964	979	48/151	−19	−22	542	678	37/150	− 9	−12	308	300	73/203	−23	−26	328	289	145/147	+ 3
78	121	− 21	− 20	984	1000	90	−61	−60	602	752	113	−37	−36	344	334	135	−68	−67	395	347	146	− 1
80	108	− 13	0	984	1000	51	−39	−26	628	785	86	−27	−14	358	348	103	−32	−19	414	364	133	− 13

Flask constant	1.42	1.75	1.36	1.23
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	390	445	−	−
Final NO_2	265	215	390	380
Final NO_3	50	40	310	320

TABLE 11

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 25°C

Time hrs.	NH_3 Reactor I a	b	c	d	e	NH_3 Reactor II a	b	c	d	e	NO_2 Reactor I a	b	c	d	e	NO_2 Reactor II a	b	c	d	e	Thermobarometer a	b
10½	169					168					185					161					15	
12½	200	+31	-11			204	+36	-6			189	+24	-18	18	19	191	+30	-12	12	15	192	+42
14½	230	+30	+5			236	+32	+7			202	+13	-12	30	31	205	+14	-11	23	28	217	+25
16½	236/151	+6	-4	4	5	245/151	+9	-1	1	1	201/150	-1	-11	41	43	207/151	+2	-8	31	38	227/150	+10
18½	139	-12	-10	14	17	147	-4	-2	3	4	136	-14	-12	53	55	142	-9	-7	38	47	148	-2
20½	127	-12	-6	20	25	139	-8	-2	5	7	123	-13	-7	60	62	133	-9	-3	41	51	142	-6
24½	120	-7	-17	37	46	140	+1	-9	14	18	120	-3	-13	73	76	122	-11	-21	62	77	152	+10
31½	53/172	-67	-36	75	93	88/171	-5	-21	35	46	68/171	-52	-21	94	98	49/170	-73	-42	104	129	121/150	-31
33½	136	-36	-11	84	104	139	-32	-7	42	55	140	-31	-6	100	104	139	-31	-6	110	136	125/	-25
35½	134	-2	-12	96	119	140	+1	-9	51	66	144	+4	-6	106	110	134	-5	-6	116	143	135	+1
39½	143	+9	-23	119	147	156	+16	-16	67	87	167	+23	-9	115	119	153	+19	-13	129	159	167	+23
41½	133	-10	-10	129	160	147	-9	-9	76	99	164	-3	-3	118	123	147	-6	-6	135	167	167	0
43½	116	-17	-11	140	173	132	-15	-9	85	111	153	-11	-5	123	128	136	-11	-5	140	173	161	-6
45½	104	-12	-14	154	191	126	-8	-10	95	123	150/156	-3	-5	128	133	132	-4	-6	146	181	163	+2
46½	98	-6	-7	161	198	120	-4	-5	100	130	167	+11	+10	138	143						164	+1
48½	93/175	-5	-12	173	214	117/180	-3	-10	110	143	173/151	+6	-1	139	145	130/165	-2	-9	155	193	171/150	+7
55½	110	-65	-55	228	282	119	-51	-41	151	196	118	-33	-23	162	168	132	-33	-23	178	220	140	-10
57½	64/180	-46	-17	245	303	76/176	-43	-14	165	214	84/177	-34	-5	167	174	97/175	-35	-6	184	228	111/165	-29

Contd...........

59½	169	-11	-15	260	322	169	- 7	-11	176	229	174	- 3	- 7	174	178	170	-5	- 9	193	238	169	+ 4
61½	172	+ 3	-19	279	346	175	+ 6	-16	192	250	187	+13	- 9	183	190	184	+14	- 8	201	249	191	+22
64½	154	-18	-26	305	376	162	-13	-21	213	278	187	0	- 8	191	199	182	- 2	-10	211	261	199	+ 8
66½	127	-27	-22	327	405	139	-23	-18	231	300	171	-11	-11	203	210	168	-14	- 9	220	272	194	- 5
69½	86	-41	-31	358	443	104	-35	-25	256	333	150	-21	-11	213	221	145	-23	-13	233	288	184	-10
72½	58/186	-28	-32	390	483	83/182	-21	-25	281	365	143/172	- 7	-11	224	233	138/150	- 7	-11	244	302	188/150	+ 4
79½	87	-99	-83	473	585	94	-88	-72	353	459	124	-48	-32	256	266	103	-47	-31	275	340	134	-16
81½	40/104	-47	-24	497	616	49/191	-45	-22	375	488	193/178	-31	- 8	264	274	69/172	-34	-11	286	354	111/186	-23
83½	181	-23	-27	524	648	170	-21	-25	400	502	175	- 3	- 7	271	282						190	+ 4

Flask constant	1.7295	1.8205	1.4515	1.7285
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	490	540	-	-
Final NO_2	180	130	485	390
Final NO_3	40	30	250	310

TABLE 12

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 28°C

Time hrs.	NH_3 Reactor I a	b	c	d	e	NH_3 Reactor II a	b	c	d	e	NO_2 Reactor I a	b	c	d	e	NO_2 Reactor II a	b	c	d	e	Thermobarometer	
11	165					168					171					181					163	
12	174	+ 9	- 0	0	0	177	+ 9	0	0	0	180	+ 9	0	0	0	190	+ 9	0	0	0	172	+ 9
14	183/178	+ 9	- 9	9	11	176/174	0	-17	17	22	192/183	+12	- 5	5	6	191/189	+ 1	-16	16	16	189/	+17
15	181	+ 3	- 4	13	16	173	- 1	- 8	25	32	188	+ 5	- 2	7	9	187	- 2	- 9	25	26	157	+ 7
17	175	- 6	- 3	16	19	155	-18	-15	40	51	181	- 7	- 4	11	14	169	-18	-15	40	41	154	- 3
19	158/172	-17	-10	26	32	134/184	-21	-14	54	69	170/185	-11	- 4	15	19	135/186	-34	-27	67	69	147/150	- 7
22½	132	-40	-23	49	60	138	-46	-29	83	107	142	-23	- 6	21	27	141	-45	-28	95	98	133	-17
24½	113/186	-19	-17	66	80	111/199	-27	-25	108	139	135/190	- 7	- 5	26	33	126/186	-15	-13	108	111	131/150	- 2
31½	90/224	-96	-64	130	158	80/236	-119	-87	195	251	147/197	-43	-11	37	47	130/193	-56	-24	132	136	118/150	-32
33½	187	-37	-24	154	187	195	-41	-28	223	287	184	-13	0	37	47	172	-21	- 8	140	144	137	-13
35½	168	-19	-18	172	209	172	-23	-22	245	313	184	0	+ 1	36	45	161	-11	-10	180	154	136	- 1
37½	149	-19	-39	211	256	152	-20	-40	285	366	198	+14	- 6	42	53	169	+ 8	-12	162	166	156	+20
39½	131	-18	-24	235	285	133	-19	-25	310	398	200	+ 2	- 4	46	58	165	- 4	-10	172	177	162	+ 6
42	89/196	-42	-38	273	332	94/207	-39	-35	345	443	191/165	- 9	- 5	51	64	148/187	-17	-13	185	190	158/150	- 4
44	152	-44	-27	300	364	169	-38	-21	366	470	150	-15	+ 2	49	62	160	-27	-10	195	200	133	-17
46	108	-44	-32	332	402	135	-34	-22	388	498	135	-15	- 3	52	66	133	-27	-15	210	216	121	-12
48	75/226	-33	-38	370	488	116/235	-11	-24	412	530	133/174	-2	- 7	59	74	123/215	-10	-15	225	231	126/150	+ 5
55	79	-147	-127	497	605	165	-70	-50	462	594	138	-36	-16	75	95	152	-63	-43	268	276	130	-20

Contd.......

57	31/223	-48	-32	529	642	143/197	-22	- 6	468	600	115/163	-23	- 7	82	103	119/169	-33	-17	285	293	114/150	-1
59	187	-36	-30	559	679	187	-10	- 4	472	606	156	- 7	- 1	83	105	151	-18	-12	297	305	144	-
61	166	-21	-27	586	711	188	+ 1	- 5	477	613	157	+ 1	- 5	88	111	142	- 9	-15	312	320	150	+
63	152	-14	-28	614	745	200	+12	- 2	479	615	161	+ 4	-10	98	124	143	+ 1	-13	325	334	164	+1
66	107	-45	-39	653	797	189	-11	- 5	484	621	141	-20	-14	112	141	110	-33	-27	352	362	158	-
68	75	-32	-19	672	815	173	-16	- 3	487	626	121	-20	- 7	119	150	81	-29	-16	368	378	145	-1
70	52/224	-23	-13	685	831	163/196	-10	0	487	626	102/196	-19	- 9	128	162	56/179	-25	-15	383	393	135/158	-1
72	212	-12	-13	698	848	192	- 4	- 5	492	632	190	- 6	- 7	135	170	163	-16	-17	400	411	159	+ 1
79	168	-44	-28	726	872	168	-24	- 8	500	641	138	-52	-36	171	216	92	-71	-55	455	466	143	-1
81	146	-22	- 9	735	890	147	-21	- 8	508	651	112	-26	-13	184	232	69	-23	-10	465	477	130	-1
83	139	- 7	- 2	737	895	139	- 8	- 3	511	656	98	-14	-11	195	246	59	-10	- 5	470	482	125	-
85	145	+ 6	- 4	741	900		Stopped				98	0	-10	205	258		Stopped				135	+1
87	152	+ 7	- 2	743	905						98	0	- 9	214	270						144	+
90	150	- 2	- 1	744	905						86	-12	-11	225	284						143	- 1
92	136	-14	- 1	745	907						72	-14	- 1	226	285						130	-1

Flask constant	1.70	1.798	1.762	1.435
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	410	490	-	-
Final NO_2	240	170	450	280
Final NO_3	50	40	250	420

TABLE 13

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 32°C

Time	NH_3 Reactor I					NH_3 Reactor II					Thermobarometer				NO_2 Reactor I					NO_2 Reactor II				
hrs.	a	b	c	d	e	a	b	c	d	e	a	b	a	b	a	b	c	d	e	a	b	c	d	e
11	204					208					151		152		218					207				
12	180	-24	-16	16	20	184	-24	-13	13	12	143	- 8	138	-14	201	-17	- 6	6	6	200				
19	182	+ 2	- 7	23	29	97	-87	-96	109	102	152	+ 9	133	- 5	157	-44	-39	45	44	182	-18	-13	13	11
21	143/204	-39	- 6	30	37	27/205	-70	-35	144	135	118/166	-33	96/166	-37	111/210	-46	-11	56	55	147/147	-35	0	13	11
23	202	- 2	- 6	36	45	175	-30	-33	177	166	170	+ 4	168	+ 2	205	- 5	- 8	64	63	148	+ 1	- 2	15	13
25	216	+14	-10	46	57	158	-17	-39	216	202	194	+24	189	+21	217	+13	-10	74	73	164	+16	- 6	21	18
27	217	+ 1	- 9	55	69	133	-25	-34	250	236	204	+10	197	+ 8	220	+ 3	- 6	80	79	168	+ 4	- 5	26	22
30	210	- 7	-12	67	84	75	-58	-61	311	291	209	+ 5	197	0	208	+12	-15	95	94	155	-13	-16	42	36
32	176/211	-34	-16	83	103	10/203	-65	-46	357	334	191/167	-18	177/165	-20	179/178	-29	-10	105	103	126/188	-20	-10	52	44
34	111	-27	-13	96	120	136	-67	-52	409	383	203	-14	148	-17	151	-27	-12	117	115	162	-26	-11	63	54
36	179/226	-12	-20	116	145	102/234	-34	-41	450	421	161/151	+ 8	154/151	+6	149/195	- 2	- 9	126	124	157/208	- 5	-12	75	64
43	172	-54	-44	160	200	76	-135	-141	591	553	141	-10	134	-17	145	-50	-33	159	156	149	-59	-42	117	100
45	150/219	-22	- 5	165	206	24/238	-52	-33	624	584	124/150	-17	113/151	-21	115/231	-30	-11	170	167	117/214	-32	-13	130	111
49	226	+ 7	-10	175	218	183	-55	-69	693	647	167	+17	163	+12	222	- 9	-23	193	190	200	-14	-28	158	134
53	243	+17	-11	186	232	165	-18	-44	737	690	195	+28	187	+24	221	- 1	-27	220	216	198	- 2	-28	186	158
55	229	-14	- 8	194	242	141	-24	-16	753	704	189	- 6	177	-10	199	-22	-14	234	230	178	-20	-12	198	168
57	211/211	-18	- 4	198	247	115/231	-26	-12	765	715	175/150	-14	162/151	-15	172/220	-27	-13	247	243	153/211	-25	-10	208	177

Contd.......

59	234	+23	-2	200	249	242	+11	-13	778	728	177	+27	172	+21	231	+11	-13	260	256	223	+12	-12	220	187
66						238	- 4	-11	789	737	196	+19	184	+12	190	-41	-56	316	310	163	-60	-75	295	250
68	Stooped					200/189	-38	- 3	792	741	162/151	-34	148/151	-36	138/189	-52	-17	333	328	108/184	-55	-20	315	268
70						150	-39	- 6	798	740	117	-34	119	-32	140	-49	-16	349	343	131	-53	-20	335	285
72						151	+ 1	0	798	748	114	- 3	113	- 6	119	-21	-17	366	360	107	-24	-20	355	302
74						180	+29	+ 1	797	747	142	+28	138	+25	130	+11	-15	381	373	112	+ 5	-21	378	320
76½						186	+ 6	0	797	747	150	+ 8	142	+ 4	118	-12	-18	399	393	89	-23	-29	405	345
78½											147	- 3	138	- 4	112	- 6	-10	409	403	65	-24	-20	425	362
80½											125	-22	116	-22	87	-25	- 3	412	405	28	-37	-15	440	374

Flask constant	1.746	1.31	1.375	1.19
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	620	470	-	-
Final NO_2	80	210	345	370
Final NO_3	-	30	360	330

TABLE 14

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 34°C

Time hrs.	NH_3 Reactor I a	b	c	d	e	NH_3 Reactor II a	b	c	d	e	NO_2 Reactor I a	b	c	d	e	NO_2 Reactor II a	b	c	d	e	Thermobarometer	
12	167					150					176					171					152	
14½	176					158					183					176					152	0
16½	219/150	+43	+11			200/151					216/150	+33	+ 1			206/150	+30	- 2	2	2	184/152	+32
18½	160	+10	+ 5			159					144	- 6	-11	11	10	141	- 9	-14	16	14	157	+ 5
20½	159	- 1	+ 6			158					117	-27	-20	31	29	116	-25	-18	34	29	150	- 7
22½	151	- 8	+ 5			148					87	-30	-17	48	45	96	-20	- 7	41	35	137	-13
26½	147/150	- 4	- 3	3	4	144/152	- 4	- 3	3	4	71/150	-16	-15	63	59	79/150	-17	-16	57	48	136/150	- 1
33½	104	-46	-25	28	36	113	-39	-18	21	26	103	-47	-26	89	83	79	-71	-50	107	90	129	-21
35½	71/169	-33	-10	38	48	83/160	-30	- 7	28	35	70/181	-33	-10	99	92	36/178	-43	-20	127	107	106/161	-26
37½	171	+ 2	- 5	43	55	160	0	- 7	35	43	184	+ 3	- 4	103	96	172	- 6	-13	140	118	168	+ 7
41½	175	+ 4	-22	65	83	173	+13	-13	48	58	198	+14	-18	115	107	158	-14	-40	180	152	194	+26
43½	162	-13	-11	76	97	164	- 9	- 7	55	68	192	- 6	- 4	119	111	134	-24	-22	202	171	192	- 2
45½	139	-23	-10	86	110	143	-21	- 8	63	78	173	-19	- 8	125	116	101	-33	-20	222	188	179	-13
47½	116	-23	-15	101	129	125	-18	-10	73	96	156	-17	- 9	134	125	68	-33	-25	247	208	171	- 8
50½	98/169	-18	-18	119	152	112/165	-13	-13	86	107	146/179	-10	-10	144	134	35/195	-33	-33	280	236	171/153	0
57½	106	-63	-55	174	222	120	-45	-37	123	153	143	-36	-28	172	160	119	-76	-68	348	294	145	- 8
59½	67/176	-39	-14	188	239	83/158	-37	-12	135	167	110/184	-33	- 8	180	168	87/187	-32	- 7	355	300	120/158	-25

Contd......

61½	168	- 8	-13	201	256	150	- 8	-13	148	184	184	0	- 5	185	172	190	+ 3	- 2	357	302	163	+ 5
63½	167	- 1	-19	220	280	156	+ 6	-12	160	198	191	+ 7	-11	196	182	199	+ 9	- 9	366	309	161	+15
64½	161	- 6	-12	232	296	156	0	- 6	166	206	192	+ 1	- 5	201	187	201	+ 2	- 4	370	312	167	+ 6
66½	139	-22	-27	259	330	146	+10	-15	181	224	185	- 7	-12	213	198	202	+ 1	- 4	374	316	192	+ 5
68½	105	-34	-25	284	362	124	-22	-13	194	240	164	-21	-12	225	209	187/174	-15	- 6	380	321	183	- 9
71½	50/166	-55	-44	328	418	89/172	-35	-24	218	270	133/16	-31	-20	245	228	179/158			387	327	172/157	-11
72½	149	-17	-10	338	430	161	-11	- 4	222	275	149	-11	- 4	249	231	148	-10	- 3	390	329	150	- 7
74½	123	-26	-28	366	466	152	- 9	-11	233	289	138	-11	-13	262	244	144	- 4	- 6	396	334	152	+ 2
81½						98/150	-54	-33	266	330	56/196	-82	-61	323	300	98/177	-46	-25	421	355	131/157	-21
83½											164	-32	-14	337	314	156	-21	- 2	425	357		

Flask constant	1.781	1.734	1.30	1.18
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	500	580	-	-
Final NO_2	160	90	425	398
Final NO_3	40	30	275	310

TABLE 15

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 36°C

Time	NO_2 Reactor I					NO_2 Reactor II					Thermobarometer				NH_3 Reactor I					NH_3 Reactor II				
hrs.	a	b	c	d	e	a	b	c	d	e	a	b	a	b	a	b	c	d	e	a	b	c	d	e
12½	152					165					153		152		174					191				
13½	177					196					176	+23	173	+21	211					228				
15½	191					206					187	+11	180	+ 7	232					256				
18	191					185					179	− 8	170	−10	232					258				
20	166					166	−19	− 2	2	2	162	−17	150	−20	234					248				
22	150	−16	− 2	2	2	149	−17	− 4	5	7	149	−13	135	−15	211					240				
24	143	− 7	− 4	6	6	142	− 7	− 5	11	13	147	− 2	130	− 5	205	− 6	− 1	2	1	237	− 3			
30	124	−19	−12	18	18	124	−18	−15	26	31	144	− 3	119	−11	180	−25	−14	15	19	220	−17	− 6	6	7
32	115	− 9	− 2	20	20	113	−11	− 4	3	36	137	− 7	112	− 7	171	− 9	− 2	17	21	206	−14	− 7	13	16
34	109	− 6	− 4	24	24	110	− 3	− 3	33	39	137	− 0	108	− 4	168	− 3	− 3	20	25	198	− 8	− 4	17	21
36	119	+10	0	24	24	117	+ 7	− 3	36	43	147	+10	117	+ 9	172	+ 4	− 5	25	31	200	+ 2	− 7	24	30
38	127	+ 8	0	24	24	125	+ 8	− 2	38	45	157	+10	123	+ 7	175	+ 3	− 4	29	37	202	+ 2	− 5	29	36
40	133	+ 6	− 2	26	26	130	+ 5	− 5	43	51	167	+10	129	+ 6	176	+ 1	− 5	34	43	200	− 2	− 8	37	46
42	125	− 8	− 2	28	28	122	− 8	− 2	45	54	161	− 6	123	− 6	163	−13	− 7	41	52	182	−18	−12	49	60
44	99	−26	− 4	32	32	97	−25	− 4	49	58	140	−21	99	−24	131	−32	− 8	49	62	142	−40	−16	65	80
46	69	−30	0	32	32	66	−31	− 0	49	58	109	−31	70	−29	95	−36	− 7	56	70	100	−42	−13	78	96
48	69/189	0	− 2	34	34	69/195	+ 2	− 4	53	63	115/161	+ 6	68/152	− 2	89/181	− 6	− 4	66	75	84/173	−16	−14	92	113
55	160	−29	− 8	42	42	162	−33	−14	67	80	142	−19	129	−23	129	−52	−29	81	113	93	−75	−52	144	177
58	146	−14	− 6	48	48	148	−14	− 8	75	89	136	− 6	118	−11	105	−24	−13	102	128	61	−37	−26	170	209

Cntd.......

60	146	0	- 8	56	56	153	+ 5	- 7	82	97	148	+12	122	+ 4	101	- 4	- 8	110	138	52/198	- 9	-13	183	225
62	152	+ 6	- 4	60	60	157	+ 4	- 8	90	107	160	+12	131	+ 9	99	- 2	-11	121	152	190	- 8	-17	200	246
66	134	-18	-12	72	72	137	-20	-15	105	125	155	- 5	124	- 7	64/217	-35	-28	149	187	141	-49	-42	242	297
68	104	-30	- 9	81	81	103	-30	-10	115	137	135	-20	102	-22	182	-35	-13	162	203	97	-44	-22	264	324
70	82	-22	- 9	90	90	87	-20	- 8	123	147	123	-12	87	-15	184	-28	-13	175	220	59	-38	-23	287	352
72	77	- 5	- 9	109	99	84	- 3	- 8	131	156	128	+ 5	89	+ 2	165	- 9	-11	186	234	36/141	-23	-25	312	393
79	27/169	-50	-37	136	136	30/182	-54	-43	174	207	117/153	-11	73/151	-16	72/188	-73	-57	243	305	46/187	-95	-79	391	480
81	181	-18	-10	146	146	164	-18	-11	185	220	146	- 7	142	- 9	167	-21	-12	255	320	158	-29	-20	411	505
83	139	-12	-14	160	160	153	-11	-13	198	236	148	+ 2	144	+ 2	153	-14	-16	270	340	133	-27	-29	440	540
85	136	- 3	-14	174	174	153	0	-14	212	252	162	+14	152	+ 8	165	- 8	-16	287	360	115	-16	-24	464	570
89½	115	-21	-32	206	206	130	-23	-35	247	294	174	+12	161	+ 9	101/198	-44	-53	340	427	54/194	-61	-70	534	655
91½	82	-33	-18	224	224	100	-30	-15	262	312	159	-15	146	-15	159	-39	-24	364	457	153	-41	-26	560	688
93½	48	-34	-16	240	240	67	-33	-14	276	328	140	-19	129	-17	118	-40	-24	388	487	109	-44	-27	587	720
96½	21	-27	-30	270	270	49	-18	-24	300	357	146	+ 6	129	0	84	-34	-34	422	530	73	-36	-36	623	765

Flask constant	1.395	1.664	1.755	1.72
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	-	-	530	450
Final NO_2	420	390	140	200
Final NO_3	280	315	30	50

TABLE 16

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 38°C

Time hrs.	NH_3 Reactor I a	b	c	d	e	NH_3 Reactor II a	b	c	d	e	NO_2 Reactor I a	b	c	d	e	NO_2 Reactor II a	b	c	d	e	Thermobarometer	
11	142					139					134					138					141	
12	181					179					169					170					162	
14	189	+ 8	+ 2			199	+20	+14			175	+ 6				152	-18	-24	24	22	168	+ 6
16	189	0	-15	13	13	198	- 1	-16	2	25	179	+ 4	-11	11	9	167	+15	0	24	22	183	+15
18	141	-48	-19	32	31	150	-48	-19	21	26	140	-39	-10	21	18	136	-31	- 2	26	24	154	-29
20	111	-30	-14	46	44	119	-31	-15	36	45	117	-23	- 7	28	23	116	-20	- 4	30	28	138	-16
22	87	-24	-21	87	84	98	-21	-18	54	68	108	- 4	- 6	34	28	113	- 3	0	30	28	135	- 3
24	59/182	-28	-16	103	99	71/173	-27	-15	69	87	90/184	-18	- 6	40	33	104/204	- 9	+ 3	27	25	123/159	-12
31	103	-79	-69	172	165	116	-57	-47	116	146	156	-28	-18	58	48	194	-10	0	27	25	149	-10
33	60/213	-43	-20	192	185	80/212	-36	-13	129	162	129/216	-27	- 4	62	52	169/169	-25	- 2	29	27	126/165	-23
35	194	-19	-19	211	103	198	-14	-14	143	179	214	- 2	- 2	64	53	168	- 1	- 1	30	28	165	0
37	189	- 5	-27	238	229	200	+ 2	-20	163	205	222	+ 8	-14	78	65	188	+20	- 2	32	29	187	+22
39	171	-18	-15	253	243	192	- 8	-11	174	218	224	+ 2	- 1	79	66	190	+ 2	- 1	33	30	190	+ 3
41	139	-32	-26	279	269	166	-26	-20	194	243	213	-11	- 5	84	70	182	- 8	- 2	35	32	184	- 6
44	76/177	-63	-39	318	306	116/179	-50	-26	220	276	171/177	-42	-18	102	85	157/170	-25	- 1	36	33	160/149	-24
48	113/186	-64	-44	362	348	122/192	-57	-37	257	322	125/194	-52	-32	134	112	148/191	-22	- 2	38	35	129/150	-20
55½	67/215	-121	-91	453	436	83/205	-109	-79	336	422	44/222	-150	-120	254	212	151/201	-40	-10	48	44	120/161	-30
57½	177	-38	-21	474	456	171	-34	-17	353	443	169	-53	-36	290	242	178	-23	- 6	54	50	144	-17
59½	159	-18	-18	493	474	155	-16	-17	370	464	136	-33	-34	324	270	174	- 4	- 5	59	54	145	+ 1

Contd.......

61½	157	- 2	-14	507	487	156	0	-12	382	480	124	-12	-24	348	290	182	+ 8	- 4	63	58	157	+12
65	119	-38	-38	545	525	123	-32	-32	414	520	72	-52	-32	400	334	172	-10	-10	73	87	157	0
67	89/201	-30	-19	564	543	94/192	-29	-18	432	542	32/194	-40	-29	429	358	153/204	-19	- 8	81	75	146	-11
70	171	-30	-18	582	560	152	-40	-28	460	576	144	-50	-38	467	390	177	-27	-15	96	89	134	-12
72	148	-23	-13	595	572	127	-25	-15	475	596	110	-34	-24	491	410	158/200	-19	- 9	105	97	124	-10
79	117/149	-31	-11	606	583	78/150	-49	-29	504	632	39/154	-71	-51	542	456	135/192	-65	-45	150	138	104/144	-20
83	136	-13	- 9	615	592	134	-16	-12	516	648	120	-34	-30	572	476	150	-42	-38	188	173	140	- 4
87	135	- 1	-12	627	604	132	- 2	-13	529	664	113	- 7	-18	590	492	124	-26	-37	225	207	151	+11

Flask constant	1.347	1.757	1.167	1.285
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	500	490	-	-
Final NO_2	165	175	270	500
Final NO_3	35	40	430	205

TABLE 17

OXYGEN UPTAKE FOR AMMONIA AND NITRITE OXIDATION AT 40°C

Time	NH_3 Reactor I					NH_3 Reactor II					NO_2 Reactor I (All Active Granules)					NO_2 Reactor II					Thermobarometer	
hrs.	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b
8	191					180					201					195					170	
9	191	0	+ 4			183					205					199					166	- 4
10	178	-13	-11	11	13	185					206					200					164	- 2
11	168	-10	- 9	20	24	181					207					201					163	- 1
12	159	- 9	- 6	26	31	180					142	-65	-62	62	61	150	-51	-48	48	51	160	- 3
19	87	-72	-59	85	103	122	-58	-45	45	56	7/199	-135	-122	184	182	37/201	-113	-100	148	174	147	-13
21	41/226	-48	-27	112	136	90/193	-32	-13	58	72	160	-39	-20	204	202	160	-41	-22	170	200	128/168	-19
22	212	-14	- 6	115	143	180	-13	- 5	63	78	142	-18	-10	214	212	142	-18	-10	180	212	160	- 8
24½	197	-15	-34	152	184	187	+ 7	-12	75	93	137	- 5	-24	238	235	137	- 5	-24	204	240	179	+19
26½	189	- 8	-22	174	211	188	+ 1	-13	88	109	133	- 4	-18	256	253	135	- 2	-16	220	259	193	+14
28½	172	-17	-24	198	240	178	-10	-17	105	130	120	-13	-20	276	273	126	- 9	-16	236	278	200	+ 7
32	111	-61	-42	240	291	134	-44	-25	130	161	65	-55	-56	312	308	77	-49	-30	266	313	181	-19
34	80	-31	-22	262	318	110	-24	-15	145	180	36	-29	-20	332	328	50	-27	-18	284	334	172	- 9
36	58/195	-22	-27	289	350	100/215	-10	-15	160	199	22/160	-14	-19	351	347	39/150	-11	-16	300	353	177/151	+ 5
42½	112	-83	-74	263	440	171	-44	-35	195	242	90	-70	-61	412	407	85	-65	-56	356	419	142	- 9
44½	77/224	-35	-22	385	467	148/165	-23	-10	205	254	57/184	-33	-20	432	426	56/166	-29	-16	372	438	129/168	-13
46½	194	-30	-20	405	492	140	-25	-15	220	273	154	-30	-20	452	446	140	-26	-16	388	456	158	-10
48½	184	-10	-26	431	524	146	+ 6	-10	230	285	152	- 2	-18	470	465	134	- 6	-22	410	482	174	+16
50½	175	- 9	-31	462	560	153	+ 7	-15	245	304	154	+ 2	-20	490	484	141	+ 7	-15	425	500	196	+22

Contd.........

52½	149	-26	-25	487	590	142	-11	-10	255	316	133	-21	-20	510	-504	122	-19	-18	443	520	195
54½	120	-29	-24	511	620	122	-20	-15	270	334	107	-26	-21	531	524	100	-22	-17	460	540	190
57½	58/247	-62	-44	555	674	89/179	-33	-15	285	353	65/168	-42	-24	555	548	58/159	-42	-24	484	569	172/160
59½	217	-30	-28	583	706	162	-17	-15	300	372	151	-17	-15	570	563	140	-19	-17	501	589	158
66½	109	-108	-99	682	826	103	-59	-50	350	434	104	-47	-38	608	600	87	-53	-44	545	640	149
69½	49/217	-60	-42	724	878	65/156	-38	-20	370	459	70/152	-34	-16	624	616	64/152	-23	- 5	550	647	131/166
71½	201	- 8	-18	742	900	151	- 5	-15	385	477	154	+ 2	- 8	632	624	160	+ 8	- 2	552	649	176
73½	190	-11	-27	769	934	152	+ 1	-15	400	496	162	+ 8	- 8	640	632						192
75	183	- 7	-13	782	949	145	- 7	-13	413	512	165	+ 3	- 3	643	635						198
78	138	-45	-47	829	1005	120	-25	-27	440	545											200

Flask constant	1.6979	1.7329	1.381	1.6449
Initial NH_3 or NO_2	700	700	700	700
Final NH_3	360	530	-	-
Final NO_2	290	150	80	70
Final NO_3	40	20	620	630

TABLE 18
OXYGEN UPTAKE FOR NITRITE OXIDATION AT 41.5°C

Time hrs.	NO_2 a	Reactor I b	c	d	e	NO_2 a	Reactor II b	c	d	e	Thermobarometer a	b
10	190					202					181	
11	192	+ 2	0	0	0	204	+ 4	+ 2	0	0	183	+ 2
13	169	-23	-27	27	36	194	-10	-14	14	13	187	+ [illegible]
15	142	-27	-33	59	56	174	-20	-25	40	36	192	+ 5
19	90	-52	-57	116	110	135	-39	-44	84	76	197	+ 5
21	67	-23	-23	139	132	118	-18	-18	101	92	197	0
24	29/238	-38	-31	170	162	87/229	-31	-24	125	114	190/192	- 7
31	171	-67	-52	222	212	163	-66	-51	176	160	177	-15
33	143	-28	-13	235	224	137	-26	-11	187	170	162	-15
35	127	-16	-13	248	236	122	-15	-12	199	181	159	- 3
38	111	-16	-21	269	256	104	-18	-23	222	202	164	+ 5
40	102	- 9	-13	282	269	95	- 9	-13	235	214	168	+ 4
42	95	- 7	-14	296	282	89	- 6	-13	248	226	175	+ 7
44	81	-14	-15	311	296	76	-13	-14	262	238	176	+ 1
46	66	-15	-17	328	312	63	-13	-15	277	252	178	+ 2
48	44/244	-22	-16	344	328	42/242	-21	-15	292	266	172/187	- 6
55	170	-74	-58	402	383	173	-69	-53	345	314	171	-15
57	151	-19	-14	416	397	153	-20	-15	360	328	166	- 5
61	115	-36	-34	450	428	115	-38	-36	396	360	164	- 2
63	98	-17	-20	470	448	100	-15	-18	414	376	167	+ 3
65	86	-12	-16	486	464	88	-12	-16	430	392	171	+ 4
68	64	-22	-28	514	489	68	-20	-26	456	416	177	+ 6
70	46	-18	-20	534	509	51	-17	-19	475	432	179	+ 2
72	23/195	-23	-21	555	529	28/188	-23	-21	496	452	177/180	- 2
79	120	-75	-65	620	590	94	-94	-84	580	528	170	-10

	Reactor I	Reactor II
Flask constant	1.332	1.272
Initial NO_2	700	700
Final NO_2	190	230
Final NO_3	510	460

APPENDIX C

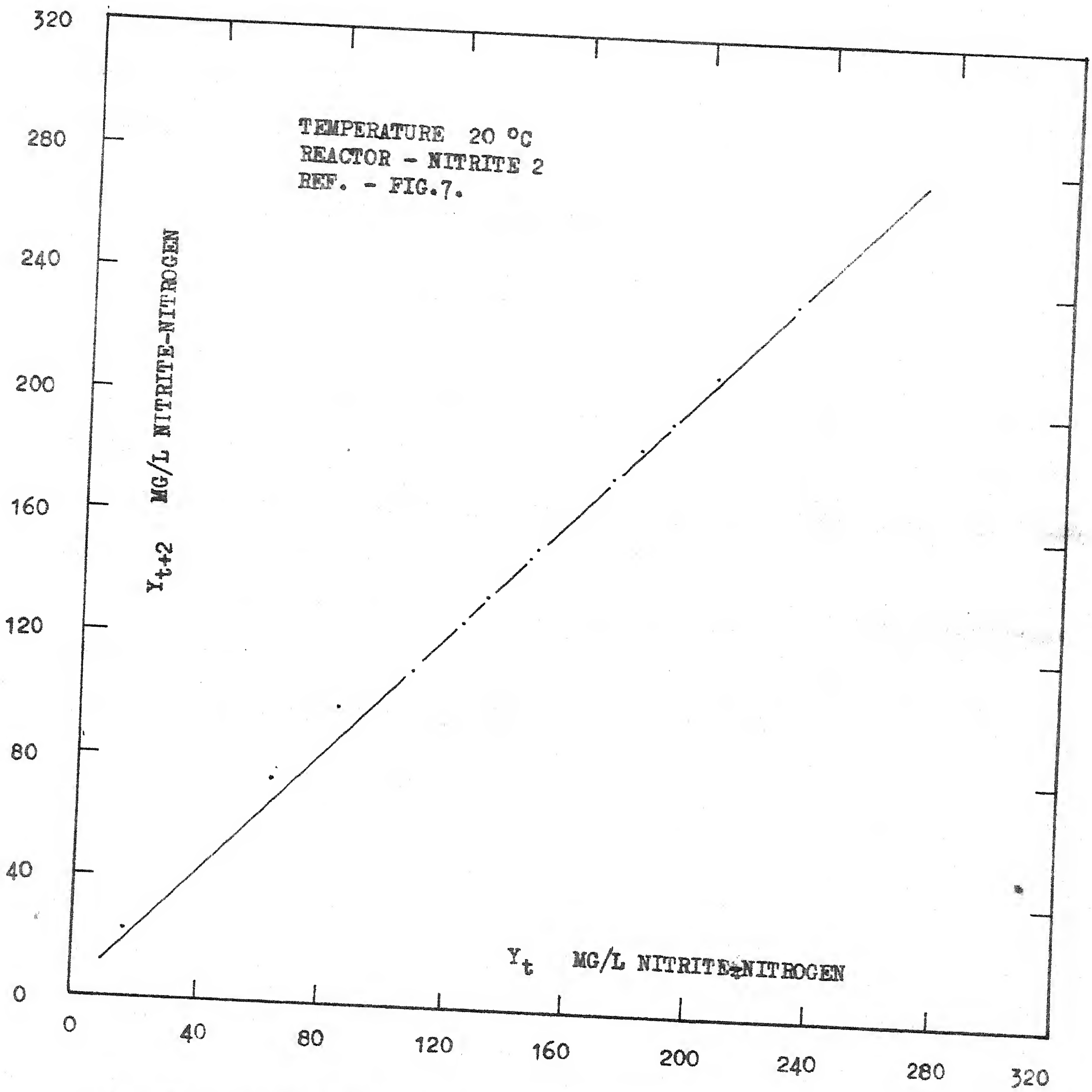

FIG. 17. DETERMINATION OF μ BY FINITE DIFFERENCE METHOD

SAMPLE CALCULATION

FOR

DETERMINING GROWTH CONSTANT

Temperature 20°C

Reactor Nitrite 2,

Reference - Fig. 17.

Slope of the straight line

$$= \frac{264 - 100}{260 - 98} = \frac{164}{162} = 1.0113$$

From equation (9),

$$\text{Slope} = e^{\mu_m . h} = 1.0113$$

so that $\mu_m . h = 0.01126$

In plotting the straight line in Fig. 17, finite interval has been taken as two hours or h = 2.

Solving for μ_m from the above equation, after substituting 2 for h,

$$\mu_m = 0.00563 \text{ hour}^{-1}$$

$$= 0.135 \text{ day}^{-1}$$